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507

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<210> 753

<211> 508

described in Verma et al., Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York (1988); and (3) Northern Blot analysis for detecting mRNA expression in specific tissues.

Preferred, however, are nucleic acid molecules having sequences at least 80%, 85%,
5 90%, 95%, 96%, 97%, 98% or 99% or 100% identical to the nucleic acid sequences disclosed herein, which do, in fact, encode a polypeptide having functional activity. By "a polypeptide having functional activity" is intended polypeptides exhibiting activity similar, but not necessarily identical, to a functional activity of the polypeptides of the present invention (e.g., complete (full-length), mature and soluble (e.g., having sequences contained in the
10 extracellular domain) as measured, for example, in a particular immunoassay or biological assay. For example, a functional activity can routinely be measured by determining the ability of a polypeptide of the present invention to bind a ligand. Functional activity may also be measured by determining the ability of a polypeptide, such as cognate ligand which is free or expressed on a cell surface, to induce cells expressing the polypeptide.

15 Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%, or 100% identical to, for example, the nucleic acid sequence of the deposited cDNA, the nucleic acid sequence shown in Table 1 (SEQ ID NO:X), or fragments thereof, will encode polypeptides "having
20 functional activity." In fact, since degenerate variants of any of these nucleotide sequences all encode the same polypeptide, in many instances, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having functional activity. This is because the skilled
25 artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid), as further described below.

For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., "Deciphering the Message in Protein Sequences:
30 Tolerance to Amino Acid Substitutions," Science 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have
5 been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For
10 example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used. (Cunningham and Wells, Science 244:1081-1085 (1989).) The resulting mutant molecules can then be tested for biological activity.

As the authors state, these two strategies have revealed that proteins are surprisingly
15 tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or
20 hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly. Besides conservative amino acid substitution, variants of the present invention
25 include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion
30 of the polypeptide with additional amino acids, such as, for example, an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification. Such variant

polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. (Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).)

A further embodiment of the invention relates to a polypeptide which comprises the amino acid sequence of a polypeptide having an amino acid sequence which contains at least one amino acid substitution, but not more than 50 amino acid substitutions, even more preferably, not more than 40 amino acid substitutions, still more preferably, not more than 30 amino acid substitutions, and still even more preferably, not more than 20 amino acid substitutions. Of course, in order of ever-increasing preference, it is highly preferable for a polypeptide to have an amino acid sequence which comprises the amino acid sequence of a polypeptide of SEQ ID NO:Y, in order of ever-increasing preference, which contains at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid substitutions. In specific embodiments, the number of additions, substitutions, and/or deletions in the amino acid sequence of SEQ ID NO:Y or fragments thereof (e.g., the mature form and/or other fragments described herein), and/or the amino acid sequence encoded by the deposited clone or fragments thereof, is 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, conservative amino acid substitutions are preferable.

Polynucleotide and Polypeptide Fragments

The present invention is also directed to polynucleotide fragments of the polynucleotides of the invention. In the present invention, a "polynucleotide fragment" refers to a short polynucleotide having a nucleic acid sequence which: is a portion of the cDNA contained in a deposited cDNA clone; or is a portion of a polynucleotide sequence encoding the polypeptide encoded by the cDNA contained in a deposited cDNA clone; or is a portion of the polynucleotide sequence in SEQ ID NO:X or the complementary strand thereto; or is a polynucleotide sequence encoding a portion of the polypeptide of SEQ ID NO:Y; or is a polynucleotide sequence encoding a portion of a polypeptide encoded by SEQ ID NO:X or

the complementary strand thereto. The nucleotide fragments of the invention are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40nt, at least about 50 nt, at least about 75 nt, or at least about 150 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from the cDNA sequence contained in a deposited clone or the nucleotide sequence shown in SEQ ID NO:X or the complementary strand thereto. In this context "about" includes the particularly recited value, a value larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. These nucleotide fragments have uses that include, but are not limited to, as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., at least 50, 150, 200, 250, 500, 600, 1000 or 2000 nucleotides in length) are also encompassed by the invention.

Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700, 701-750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, 2001-2050, 2051-2100, 2101-2150, 2151-2200, 2201-2250, 2251-2300, 2301-2350, 2351-2400, 2401-2450, 2451-2500, 2501-2550, 2551-2600, 2651-2700, 2701-2750, 2751-2800, 2800-2850, 2851-2900, 2901-2950, 2951-3000, 3001-3050, 3051-3100 and 3101 to the end of SEQ ID NO:X, or the complementary strand thereto, or the cDNA contained in the deposited clone. In this context "about" includes the particularly recited ranges, and ranges larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has biological activity. More preferably, these polynucleotides can be used as probes or primers as discussed herein. Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-

400, 401-450, 451-500, 501-550, 551-600, 651-700, 701-750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, 2001-2050, 2051-2100, 2101-2150, 2151-2200, 2201-2250, 2251-2300, 2301-2350, 2351-2400, 2401-2450, 2451-2500, 2501-2550, 2551-2600, 2601-2650, 2651-2700, 2701-2750, 2751-2800, 2801-2850, 2851-2900, 2901-2950, 2951-3000, 3001-3050, 3051-3100 and 3101 to the end of the cDNA nucleotide sequence contained in the deposited cDNA clone, or the complementary strand thereto. In this context "about" includes the particularly recited range, or a range larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has a functional activity (e.g., biological activity) of the polypeptide encoded by the cDNA nucleotide sequence contained in the deposited cDNA clone. More preferably, these fragments can be used as probes or primers as discussed herein. Polynucleotides which hybridize to one or more of these fragments under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention, as are polypeptides encoded by these polynucleotides or fragments.

In the present invention, a "polypeptide fragment" refers to an amino acid sequence which is a portion of that contained in SEQ ID NO:Y, encoded by SEQ ID NO:X or the complement thereof and/or encoded by the cDNA contained in the deposited clone. Protein (polypeptide) fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 102-120, 121-140, 141-160, 161-180, 181-200, 201-220, 221-240, 241-260, 261-280, 281-300, 301-320, 321-340, 341-360, 361-380, 381-400, 401-420, 421-440, 441-460, 461-480, 481-500, 501-520, 521-540, 541-560, 561-580, 581-600, 601-620, 621-640, 641-660, 661-680, 681-700, 701-720, 721-740, 741-760, 761-780, 781-800, 801-820, 821-840, 841-860 and 861 to the end of SEQ ID NO:Y. Moreover, polypeptide fragments can be about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges or values, and ranges or values larger or smaller by several (5, 4, 3,

2, or 1) amino acids, at either extreme or at both extremes. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Even if deletion of one or more amino acids from the N-terminus of a protein results in modification or loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind a ligand) may still be retained. For example, the ability of shortened muteins to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptides generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a mutein with a large number of deleted N-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

Accordingly, polypeptide fragments include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotides encoding these polypeptide fragments are also preferred.

The present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of a polypeptide disclosed herein (e.g., a polypeptide of SEQ ID NO:Y, and/or a polypeptide encoded by the cDNA contained in a deposited clone). In particular, N-terminal deletions may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y), and m is defined as any integer ranging from 2 to q-6. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind a ligand) may still be retained. For example the ability of the shortened mutein to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a mutein with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

Accordingly, the present invention further provides polypeptides having one or more residues from the carboxy terminus of the amino acid sequence of a polypeptide disclosed herein (e.g., a polypeptide of SEQ ID NO:Y, a polypeptide encoded by the polynucleotide sequence contained in SEQ ID NO:X, and/or a polypeptide encoded by the cDNA contained in deposited cDNA clone referenced in Table 1). In particular, C-terminal deletions may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of an amino acid residue in a polypeptide of the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

In addition, any of the above described N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of a polypeptide encoded by SEQ ID NO:X (e.g., including, but not limited to the preferred polypeptide disclosed as SEQ ID NO:Y), or the cDNA contained in a deposited clone, and/or the complement thereof, where n and m are integers as described above. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Any polypeptide sequence contained in the polypeptide of SEQ ID NO:Y, encoded by the polynucleotide sequences set forth as SEQ ID NO:X or the complement thereof, or encoded by the cDNA in the related cDNA clone contained in the deposit may be analyzed to

determine certain preferred regions of the polypeptide. For example, the amino acid sequence of a polypeptide encoded by a polynucleotide sequence of SEQ ID NO:X or the complement thereof, or the cDNA in a deposited cDNA clone may be analyzed using the default parameters of the DNASTAR computer algorithm (DNASTAR, Inc., 1228 S. Park St., Madison, WI 53715 USA; <http://www.dnastar.com/>).

Polypeptide regions that may be routinely obtained using the DNASTAR computer algorithm include, but are not limited to, Garnier-Robson alpha-regions, beta-regions, turn-regions, and coil-regions, Chou-Fasman alpha-regions, beta-regions, and turn-regions, Kyte-Doolittle hydrophilic regions and hydrophobic regions, Eisenberg alpha- and beta-amphipathic regions, Karplus-Schulz flexible regions, Emini surface-forming regions and Jameson-Wolf regions of high antigenic index. Among highly preferred polynucleotides of the invention in this regard are those that encode polypeptides comprising regions that combine several structural features, such as several (e.g., 1, 2, 3 or 4) of the features set out above.

Additionally, Kyte-Doolittle hydrophilic regions and hydrophobic regions, Emini surface-forming regions, and Jameson-Wolf regions of high antigenic index (i.e., containing four or more contiguous amino acids having an antigenic index of greater than or equal to 1.5, as identified using the default parameters of the Jameson-Wolf program) can routinely be used to determine polypeptide regions that exhibit a high degree of potential for antigenicity. Regions of high antigenicity are determined from data by DNASTAR analysis by choosing values which represent regions of the polypeptide which are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the process of initiation of an immune response.

Preferred polypeptide fragments of the invention are fragments comprising, or alternatively consisting of, an amino acid sequence that displays a functional activity of the polypeptide sequence of which the amino acid sequence is a fragment.

By a polypeptide demonstrating a "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a polypeptide for binding) to an anti-polypeptide antibody], immunogenicity (ability to generate antibody which binds to

a specific polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide.

Other preferred polypeptide fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

In preferred embodiments, polypeptides of the invention comprise, or alternatively consist of, one, two, three, four, five or more of the antigenic fragments of the polypeptide of SEQ ID NO:Y, or portions thereof. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Table 8

Contig ID/ Sequence ID	Epitopes
390631	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4278 as residues: Asn-1 to Asn-6.
410299	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4279 as residues: Trp-26 to Met-31.
456200	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4280 as residues: Pro-16 to His-26, Arg-45 to Gly-51.
471563	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4283 as residues: Gly-37 to Glu-47.
488131	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4284 as residues: Met-26 to Leu-32, Gly-41 to Asn-46.
500696	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4286 as residues: Lys-16 to Glu-31, Ser-47 to Glu-54.
506406	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4288 as residues: Thr-110 to Tyr-118.
506619	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4289 as residues: Cys-50 to Phe-57, Phe-69 to Asp-76, Ser-89 to Gln-104, Glu-145 to Leu-153.
507852	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4290 as residues: Glu-8 to Trp-18, Arg-46 to Ala-51.
509423	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4291 as residues: Tyr-50 to Ser-56, His-58 to Tyr-65.
524721	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4294 as residues: Pro-1 to Ser-8.
524901	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4295 as residues: Leu-34 to Lys-39, Lys-57 to Gly-63.
527600	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4296 as residues: Val-28 to Gly-34, His-57 to His-63.
529050	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4298 as residues: Asn-2 to Lys-8.
529465	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4299 as residues: Ala-12 to Gln-24.
532810	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4302 as residues: Pro-1 to Trp-7, Glu-124 to Trp-130.
541126	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4304 as residues: Thr-1 to Asn-10, Ala-72 to Gly-77, Val-84 to Gly-90.
542268	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4305 as residues: Pro-34 to Pro-40, Pro-45 to Ser-50, Gly-73 to Gly-82.
547920	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4306 as residues: Pro-28 to Thr-35.
552465	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4310 as residues: Pro-4 to Gly-10, Thr-17 to Leu-29, Pro-53 to Gly-58, Gln-78 to Lys-86, Pro-88 to Lys-94, His-137 to Gly-142.

554369	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4311 as residues: Gln-20 to Gln-27.
557152	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4312 as residues: Ser-69 to Pro-74.
557230	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4313 as residues: Pro-21 to Cys-31, Val-34 to Gly-42.
570796	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4315 as residues: Glu-34 to Ala-39.
573181	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4316 as residues: Gly-4 to Arg-11, Gly-17 to Ala-24.
573793	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4318 as residues: Glu-4 to Ser-9.
573796	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4319 as residues: Pro-4 to Asn-13, Asn-57 to Arg-66, Pro-89 to Asn-99.
574927	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4321 as residues: Asp-1 to Ile-6, Pro-37 to Gln-42, Pro-61 to Trp-68.
575139	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4322 as residues: Met-2 to Asp-9.
575591	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4323 as residues: Ala-2 to Gly-11.
577390	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4325 as residues: Glu-53 to Leu-58, Gln-60 to Glu-65.
577685	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4326 as residues: Ile-5 to Gln-12, Leu-42 to Asn-51.
578660	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4328 as residues: His-1 to Phe-6, Val-11 to Arg-23.
580860	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4329 as residues: Ser-14 to Asn-22.
581143	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4330 as residues: Ile-1 to Gly-6.
584899	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4331 as residues: Ala-29 to Asn-35.
600669	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4332 as residues: Cys-1 to Ala-18, Cys-55 to Ile-61.
611839	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4333 as residues: Arg-35 to Gly-41.
614078	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4334 as residues: Glu-8 to Leu-14.
630230	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4338 as residues: Arg-77 to Lys-83.
637605	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4340 as residues: Ser-1 to Val-11.
638125	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4341 as residues: Ser-7 to Glu-12, Pro-20 to Ser-26, Arg-31 to Glu-43, Ala-69 to Glu-80, Val-90 to His-95, Pro-100 to Ser-107, Ser-109 to Glu-115, Ala-117 to Arg-124.
638249	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 4343 as residues: Asp-1 to Pro-28, Gln-73 to Ser-79, Ile-91 to Gly-96, Tyr-99 to Asp-109, Gln-183 to Pro-193, Val-249 to Thr-261.
638319	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4344 as residues: Gly-23 to Gly-28, Asp-35 to Gln-53.
651380	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4345 as residues: Thr-16 to Lys-35, Lys-46 to Arg-51.
651876	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4346 as residues: Arg-1 to Asp-12, Pro-25 to Ala-34, Ala-50 to Gly-55, Glu-66 to Lys-86.
653175	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4347 as residues: Thr-45 to Asn-50.
655544	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4348 as residues: Arg-2 to Asp-18, Leu-45 to Leu-51.
656722	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4349 as residues: Gln-21 to Leu-38.
659801	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4350 as residues: Gly-2 to Gly-20, Pro-45 to Ala-51, Glu-105 to Gln-112, Gln-117 to Glu-122, Ala-207 to Leu-215.
660020	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4351 as residues: Ser-40 to Thr-52.
664481	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4353 as residues: Gly-1 to Glu-15, Phe-20 to Tyr-25, Phe-53 to Asn-58, Glu-82 to Lys-93.
665154	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4354 as residues: Pro-18 to Arg-29.
668040	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4356 as residues: Glu-5 to Ala-14, Arg-69 to Ala-76, Ala-114 to Glu-120, Ser-132 to Leu-137.
668717	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4358 as residues: Arg-3 to Gly-12, Ala-51 to Asp-65, Leu-78 to Glu-84, Arg-118 to Asp-131, Leu-157 to Asn-168.
671361	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4360 as residues: Asn-1 to Ser-6, Glu-15 to Gln-20.
674203	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4361 as residues: Gly-7 to Ile-13.
674745	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4362 as residues: Val-17 to Arg-26, Lys-38 to Leu-48, Gln-129 to Trp-136, Gln-258 to Leu-263, Ala-272 to Glu-284, Pro-380 to Asp-391.
674761	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4363 as residues: Ala-14 to His-19.
677212	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4364 as residues: Gly-1 to Ser-14, Asn-29 to Trp-34, Lys-50 to Arg-60.
685895	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4366 as residues: Arg-28 to Ser-33.
688040	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4367 as residues: Thr-2 to Ser-7, Pro-132 to Asp-138, Ile-161 to Pro-170, Pro-212 to Asn-217, Gly-280 to Gln-313, Ser-332 to His-337,

	Asn-366 to Gly-372.
688044	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4368 as residues: Asn-33 to Pro-55, Lys-67 to Arg-74, Gly-85 to Tyr-94, Arg-101 to Pro-115, Ser-123 to Cys-129, Pro-155 to Val-162, Pro-172 to Cys-184.
691124	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4370 as residues: Pro-27 to Arg-35.
691721	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4371 as residues: Lys-23 to Gln-29, Gly-59 to Asn-77.
693582	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4372 as residues: Lys-12 to Lys-17.
696007	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4373 as residues: Gln-93 to Arg-101, Tyr-104 to Thr-113, His-134 to Gln-145, Ser-154 to Gln-165, Val-231 to Pro-248.
703700	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4377 as residues: Lys-1 to Ser-21.
705461	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4378 as residues: Ala-53 to Glu-59, Thr-69 to Gln-77, Glu-107 to Trp-114.
707464	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4382 as residues: Glu-1 to Tyr-14, Lys-41 to Arg-51, Thr-54 to Arg-73, Gly-77 to Thr-84, Thr-92 to Ser-100, Gln-107 to Arg-112, Ala-114 to Ser-141.
709015	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4383 as residues: Pro-62 to Ser-67.
711878	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4387 as residues: Ser-3 to Lys-10.
712638	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4388 as residues: Leu-31 to His-36, Val-94 to Phe-105.
715343	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4392 as residues: Phe-7 to Ile-12, Leu-17 to Ser-24.
716212	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4393 as residues: Ser-1 to Trp-6, Pro-8 to Pro-21, Arg-60 to Asp-65, Tyr-70 to Lys-80, Lys-116 to Met-121.
717222	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4394 as residues: Glu-40 to Ala-45, Pro-66 to Ser-80, Gly-99 to Ala-107.
719829	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4396 as residues: Leu-15 to Cys-20.
721985	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4397 as residues: Asp-1 to Leu-19.
722249	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4398 as residues: Ala-54 to Gly-59, Ser-67 to Gly-78, Ala-131 to Pro-136, Pro-151 to His-157, Pro-172 to Asn-181, His-183 to Gln-192, Ala-200 to Asn-208, Thr-220 to Ile-226, Glu-335 to Arg-341, Ser-397 to Cys-404, Lys-415 to Phe-423, Lys-432 to Leu-437.
722258	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4399 as residues: Trp-15 to Ala-24, Arg-38 to Glu-45, Tyr-51 to Gly-59.

725110	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4401 as residues: Leu-23 to Asn-32.
725201	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4402 as residues: Asn-1 to Ser-9, Leu-49 to Leu-64, Leu-68 to Arg-73, Lys-83 to Thr-90.
727365	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4404 as residues: Val-36 to Lys-51, Asn-59 to Asn-76, Val-91 to Lys-107, Leu-112 to Cys-135, Arg-140 to Lys-150, Pro-157 to Glu-173, Thr-188 to Lys-201, Lys-207 to Ile-226, Leu-234 to Thr-258, Glu-260 to Ile-268, Ser-275 to Lys-286, Val-288 to Glu-299.
731881	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4407 as residues: Lys-8 to His-18.
734012	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4411 as residues: Lys-34 to Ser-39.
735603	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4412 as residues: His-1 to Gln-6, Glu-19 to Val-26.
739061	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4413 as residues: Asn-7 to Lys-13.
741134	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4414 as residues: Pro-10 to Trp-18.
741804	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4416 as residues: Asp-21 to Ser-30, His-37 to Lys-48, Phe-75 to Arg-82.
742220	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4417 as residues: Val-17 to Pro-23, Ser-72 to His-79, Thr-93 to Ile-100, Pro-102 to Asp-108, Asn-111 to Tyr-117, Gly-134 to Lys-141.
744605	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4418 as residues: Asp-1 to Lys-11.
745368	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4420 as residues: Lys-10 to Ser-16, Pro-30 to Arg-37.
750486	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4422 as residues: Asp-21 to Asp-28, Ser-34 to Asp-40.
751119	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4423 as residues: Gly-1 to Gly-13, Gly-18 to Glu-29.
753226	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4425 as residues: Asp-1 to Arg-9, Asn-51 to Cys-57, Cys-125 to Leu-137, Cys-153 to Trp-166, Leu-181 to Glu-186, Ser-207 to Thr-212.
756466	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4427 as residues: Ser-1 to Asn-8.
756649	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4429 as residues: Gly-1 to His-10, His-21 to Asp-32.
757213	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4430 as residues: Ala-17 to Leu-23, Gly-28 to Gly-42, His-55 to Glu-62, Gly-92 to Ala-100.
757508	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4431 as residues: Ser-23 to Arg-32, Glu-39 to Thr-45, Glu-52 to Lys-57.
757980	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4433 as residues: Phe-9 to His-21.

760141	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4434 as residues: Ser-15 to Gly-21, Asp-35 to His-41, Glu-45 to Lys-68, Thr-91 to Trp-103, Glu-105 to Gln-116, Asp-124 to Gly-130, Asp-137 to Thr-147, Glu-162 to Gly-188, Lys-205 to Gly-212, Asn-223 to Trp-229, Arg-241 to Lys-254.
761491	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4435 as residues: Gly-55 to Glu-63.
764179	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4438 as residues: Asn-1 to Thr-7.
766961	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4439 as residues: Leu-5 to Glu-16.
768034	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4441 as residues: Ser-20 to Lys-29.
769965	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4442 as residues: Asn-1 to Ser-9, Pro-11 to Cys-38, Pro-41 to Val-46, Trp-55 to Ser-62, Pro-73 to Phe-78, Leu-97 to Gln-103, Arg-110 to Gly-116.
771486	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4443 as residues: Glu-16 to Lys-21.
772044	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4444 as residues: Ala-11 to Ala-23.
772357	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4445 as residues: Phe-61 to Glu-66.
772876	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4446 as residues: Arg-80 to Thr-91.
774019	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4447 as residues: Ser-1 to Cys-9, Gln-22 to Gln-28, Gly-41 to Gly-47, Leu-57 to Arg-66.
774516	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4449 as residues: Leu-41 to Gln-48.
775355	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4450 as residues: Ser-40 to Ala-46.
775367	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4451 as residues: Lys-8 to Lys-28.
775791	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4452 as residues: Arg-19 to Asp-29, Asn-81 to Lys-86.
778583	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4455 as residues: Thr-10 to Trp-16, Gly-41 to Phe-46, Ser-55 to Phe-65.
779588	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4457 as residues: Leu-19 to Lys-26.
781085	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4458 as residues: Ala-57 to Ser-64, Lys-69 to Thr-75.
781366	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4460 as residues: Arg-24 to Pro-35, Gly-72 to His-77.
781376	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4461 as residues: Pro-39 to Cys-44, Pro-54 to Gly-65.
782276	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4463 as residues: Ile-1 to Gln-9, Arg-27 to Pro-34, Val-36 to

	Pro-60, Lys-86 to Asp-95, Lys-102 to Ser-113, Ser-118 to Asn-130, Asp-132 to Lys-143, Asp-151 to Glu-157.
783413	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4465 as residues: Lys-33 to Val-39.
783668	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4466 as residues: Gly-8 to Leu-17, Leu-27 to Ser-36, Pro-41 to Ser-51.
785087	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4468 as residues: Lys-26 to Lys-42.
785465	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4470 as residues: Gly-6 to Arg-21.
788626	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4471 as residues: Leu-1 to Lys-21, Asp-26 to Asp-34, Ala-85 to Tyr-90.
788838	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4472 as residues: Ala-14 to Ile-19, Glu-48 to Glu-54, Gln-76 to Glu-89.
789419	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4474 as residues: Pro-16 to Asn-22.
789631	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4475 as residues: Thr-10 to Gly-18.
789872	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4476 as residues: Ser-1 to Phe-16, His-36 to Gly-45, Pro-49 to Pro-71, Pro-77 to Lys-84.
790190	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4477 as residues: Ser-41 to Thr-49.
790547	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4478 as residues: Leu-1 to Gln-19, Glu-24 to Pro-31, Lys-36 to Cys-45.
792557	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4483 as residues: Lys-51 to Arg-58.
792624	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4484 as residues: Ser-15 to Lys-22, Pro-25 to Gly-47, Glu-55 to Thr-64.
793437	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4485 as residues: Pro-1 to Gly-7, Thr-9 to Phe-18, Ala-32 to Trp-45, Pro-53 to Leu-60, Thr-66 to Arg-71.
796023	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4488 as residues: Ala-69 to Cys-74, Ile-131 to Glu-136, Gly-161 to Asn-169, Leu-174 to Trp-185.
796181	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4489 as residues: Ser-26 to Arg-32, Ala-81 to Cys-87, Pro-118 to Lys-126.
797079	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4490 as residues: Phe-2 to Cys-8, Ser-30 to His-36.
797477	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4491 as residues: Gly-14 to Leu-24.
797486	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4492 as residues: Ser-18 to Gln-25, Pro-35 to Thr-44, Pro-94 to

	Trp-99, Gln-108 to Ser-120, Pro-182 to Gly-187, Pro-192 to Gly-198, Trp-284 to Thr-292.
797747	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4493 as residues: Asn-2 to Ala-11, His-35 to Pro-40.
805448	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4496 as residues: Leu-1 to Tyr-7, Gly-15 to Asn-26.
806690	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4497 as residues: Gly-34 to Trp-43, Trp-48 to Lys-54.
810870	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4498 as residues: Val-12 to Ile-21.
811047	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4499 as residues: Phe-8 to Gly-13, Glu-16 to Asn-34, Ser-179 to Cys-185, Thr-206 to Phe-219.
812745	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4500 as residues: Gly-50 to His-62, Lys-169 to Arg-174, Thr-200 to Asp-206, Leu-208 to Gly-214, Pro-244 to Glu-254, Asp-304 to Gln-310, Gln-318 to Trp-323, Thr-410 to His-415.
812871	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4502 as residues: Ser-22 to Arg-29.
813482	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4503 as residues: Cys-53 to His-65, Glu-71 to Gln-91, Asn-123 to Phe-131, Ala-157 to Pro-171, Gln-197 to Ala-238.
815696	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4504 as residues: Arg-80 to Glu-86, Pro-102 to Thr-110, Pro-113 to Phe-122, Asn-124 to Tyr-131, Thr-149 to Cys-156, Thr-184 to Pro-196, Ser-203 to Cys-215, Gly-226 to Asp-231, Pro-285 to Gly-290.
821335	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4505 as residues: Ser-47 to Cys-59.
827315	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4508 as residues: Asp-29 to Phe-36, Phe-39 to Gly-51.
827740	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4511 as residues: Ile-22 to Lys-28.
828180	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4512 as residues: Glu-38 to Arg-52, Ser-56 to Val-62.
828552	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4513 as residues: Ser-1 to Ser-10, Leu-64 to Asp-69, Gly-102 to Arg-107.
828919	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4515 as residues: Thr-49 to Val-54, Leu-83 to Lys-91, Gly-121 to Thr-130, Asp-165 to Glu-172, Thr-180 to Gly-188.
829084	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4516 as residues: Glu-37 to Trp-47.
829148	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4517 as residues: Pro-33 to Lys-40.
829161	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4518 as residues: Met-5 to Glu-18, Asp-24 to Tyr-30.
830123	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4519 as residues: Ala-20 to Arg-25.
830194	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 4521 as residues: Ala-43 to Lys-51, Glu-66 to Leu-74, His-81 to Glu-88, Arg-98 to Ser-105, Gly-111 to Gln-116, Leu-166 to Lys-182, Leu-261 to Ala-273, Glu-294 to Arg-302, Glu-335 to Asp-347.
830343	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4524 as residues: Ser-19 to Gly-24, Lys-73 to Leu-94, Ala-101 to Arg-112, Gly-137 to Ala-143, Glu-160 to Arg-168, Ser-173 to Lys-183.
830347	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4525 as residues: Asp-33 to Ala-39.
830382	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4526 as residues: Leu-47 to Val-63, Ser-69 to Ser-76.
830465	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4528 as residues: Pro-1 to Thr-8, Ser-54 to Gln-61, Thr-80 to Thr-85, Gln-92 to Tyr-98, Gln-154 to Gln-162, Glu-172 to Ile-177, Val-181 to Lys-188, Lys-213 to Asn-225, Ser-234 to Pro-239, Ile-294 to Lys-307, Gly-350 to Asn-355.
830498	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4529 as residues: Pro-39 to Asn-47.
830540	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4530 as residues: Leu-31 to Lys-37, Arg-48 to Asn-54.
830586	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4533 as residues: Pro-1 to Gln-15, Arg-33 to Leu-40, Arg-72 to Ser-78, Leu-98 to Asp-103, Phe-116 to Gly-124, Pro-152 to Arg-158, Thr-193 to Pro-200, Leu-213 to Phe-219, Asp-229 to Lys-237, Lys-246 to Lys-258, Arg-275 to Thr-280, Thr-306 to Lys-312, Leu-320 to Arg-328, Ala-335 to Asn-340, Gly-342 to Trp-349, Cys-364 to Pro-372.
830693	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4535 as residues: Met-2 to Thr-12, Gln-52 to Glu-67, Glu-72 to Val-79, Asn-158 to Arg-165, Met-173 to Gln-180, Glu-200 to Arg-206, Ala-220 to Ala-228, Arg-232 to Leu-242, Asp-246 to Gln-254, Thr-260 to Lys-267, Leu-343 to Glu-349.
830723	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4537 as residues: Ile-68 to Thr-75, Asp-106 to Asp-117.
830743	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4538 as residues: Pro-11 to Phe-16, Thr-48 to Ser-60.
830804	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4539 as residues: Thr-62 to Gly-70.
830816	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4540 as residues: Thr-51 to Asp-61, Pro-92 to Asn-100, Thr-131 to Asn-138, Lys-140 to His-151, Glu-168 to Arg-184, Glu-192 to Glu-197, Ala-202 to Leu-212, Tyr-218 to Lys-223, Ala-239 to Leu-246, Leu-250 to Gly-256, Pro-289 to Glu-295, Lys-314 to Lys-326, Gln-335 to Glu-340, Asp-354 to Ser-359.
830829	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4541 as residues: Pro-16 to His-21, Cys-28 to His-35, Val-43 to Arg-49, Pro-116 to Tyr-123.
830859	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4542 as residues: Gln-13 to His-28, Pro-73 to Gly-80, Pro-87 to Asn-92.
830879	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 4543 as residues: Cys-34 to Leu-44, Ser-60 to Gly-69, Asp-118 to Gly-123, Cys-148 to Gln-154.
830901	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4544 as residues: Arg-8 to Ser-16, Val-32 to Thr-38, Glu-139 to Lys-145, Arg-224 to Arg-232.
831019	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4545 as residues: Phe-16 to Ser-21.
831057	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4546 as residues: Arg-1 to Gly-14, Thr-19 to Gly-25, Ala-31 to Ala-41, Glu-53 to Ile-62, Val-66 to Glu-75, Ser-103 to Asp-113, Ala-135 to Asp-140.
831099	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4547 as residues: Leu-12 to Gly-18, Leu-93 to Ile-98, Lys-165 to Ser-183, Thr-198 to Lys-211, Glu-232 to Gly-237, Pro-239 to Gly-249, Arg-257 to Asp-278, Cys-292 to Glu-297, Arg-306 to Ser-316, Asp-323 to Asn-331, Glu-347 to Gly-354, Thr-365 to Asn-370, Pro-390 to Thr-396, Asn-420 to Ser-433, Val-440 to Gln-451, His-457 to Asp-465, Phe-533 to Met-538, Ala-540 to Tyr-550, Pro-560 to Lys-565.
831117	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4548 as residues: Lys-50 to Tyr-55.
831163	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4549 as residues: Ser-31 to Arg-40.
831212	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4551 as residues: Arg-34 to Gly-45, Pro-50 to Ala-58.
831234	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4552 as residues: Gly-28 to Pro-33, Gln-66 to Gln-72.
831268	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4554 as residues: Ser-16 to Lys-21.
831307	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4555 as residues: Pro-19 to Ile-26, Ala-43 to Thr-49, Ser-52 to Lys-69, Phe-126 to Arg-134, Pro-153 to Phe-161, Ser-192 to Leu-198, Arg-222 to Thr-229.
831390	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4558 as residues: Trp-50 to Gly-55, Leu-109 to Val-119, Phe-146 to Asp-158, Ser-165 to Trp-172, Phe-192 to Ile-197, Leu-241 to Asp-252, Lys-268 to Pro-273, Ser-310 to Lys-315, Asp-334 to Ala-342, Pro-348 to Tyr-353.
831426	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4559 as residues: Gly-8 to Phe-18, His-26 to Phe-41, Glu-56 to Gly-62, Phe-114 to Lys-126, Asn-198 to Ser-203, Asn-234 to Ile-242, Glu-264 to Pro-270.
831453	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4560 as residues: Tyr-34 to His-42, Leu-44 to Leu-49.
831465	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4561 as residues: Thr-2 to Ser-9, Pro-23 to Ser-28, Phe-55 to Ala-60, Phe-72 to Ile-77, Leu-124 to Gly-136, Glu-138 to Val-144.
831586	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4563 as residues: Gln-14 to Glu-28.
831664	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4564 as residues: Lys-1 to Asp-42, Arg-71 to Ala-76, Gln-138

	to Phe-145, Lys-170 to Thr-178, Cys-186 to Asp-192.
831687	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4565 as residues: Ala-56 to Tyr-63.
831753	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4567 as residues: His-10 to Gly-16, Gly-30 to Phe-36, Ala-41 to Lys-47, Phe-63 to Trp-72.
831757	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4568 as residues: Val-81 to Lys-86.
831795	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4569 as residues: Asn-23 to Pro-28, Arg-36 to Ser-42.
831796	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4570 as residues: Pro-1 to Ser-8.
831880	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4571 as residues: Asp-18 to Ser-24, His-34 to Gly-47.
831899	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4572 as residues: Asp-11 to Trp-16, Pro-37 to Thr-44, Pro-74 to Pro-82, Arg-112 to Gln-119, Cys-126 to Arg-138, Arg-199 to Thr-204.
831910	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4573 as residues: Gly-15 to Trp-21, Ser-84 to Leu-93.
831931	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4574 as residues: Asn-29 to Ser-34.
831942	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4575 as residues: Arg-14 to Trp-19, Pro-29 to Gly-37, Cys-51 to Ala-62, Glu-84 to Glu-91, Ile-101 to Pro-107, Glu-118 to Thr-123, Lys-170 to Gln-175, Thr-197 to Lys-228.
832009	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4577 as residues: Leu-17 to Arg-32.
832010	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4578 as residues: Leu-1 to Lys-21, Glu-39 to Cys-47, Lys-49 to Gln-61, His-64 to Gly-76, Thr-83 to Lys-90, His-92 to Ile-99.
832093	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4580 as residues: Pro-29 to Tyr-35, Phe-37 to His-42.
832187	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4583 as residues: Glu-11 to Pro-24, Gly-90 to Leu-96, Ser-109 to Gly-120.
832575	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4588 as residues: Thr-24 to Arg-29, Ala-55 to Tyr-60, Tyr-77 to Asp-89, Leu-108 to Gly-115, Thr-142 to Gly-149.
832593	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4589 as residues: Glu-13 to Glu-18.
832597	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4590 as residues: Val-3 to Asp-13.
834890	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4591 as residues: Arg-8 to Lys-13, Gly-35 to Lys-42, Ala-48 to Lys-54, Ala-105 to Leu-110, Gly-150 to Val-157, Phe-164 to Asn-173.
835079	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4592 as residues: Ser-53 to Pro-60.
835456	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4593 as residues: Thr-2 to Asn-10, Ser-72 to Lys-78, Gly-95 to

	Thr-101, Phe-134 to Ile-147, Lys-163 to Lys-172, Gln-199 to Glu-206, Ala-212 to Trp-224, Lys-230 to His-236, Arg-238 to Glu-244, Asp-249 to Gly-254, Met-260 to Tyr-266, Arg-272 to Arg-279.
835655	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4594 as residues: Lys-24 to Asn-36, Glu-55 to Asn-60.
836203	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4595 as residues: Pro-43 to Cys-49, Ser-67 to Glu-76, Lys-105 to Cys-110.
836762	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4597 as residues: Arg-252 to Phe-260, Ser-315 to Thr-321.
838459	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4600 as residues: Asp-1 to Lys-14.
839262	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4601 as residues: Lys-29 to Asp-36, Gln-98 to Asp-103, Thr-120 to Lys-142, Thr-158 to Ser-170, Ile-188 to Glu-194, Leu-217 to Gly-223, Tyr-245 to His-252.
839750	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4603 as residues: Gln-27 to Pro-33.
840028	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4604 as residues: Ala-16 to Asn-25, His-32 to Asn-37, Pro-97 to Ser-103, Pro-114 to Ser-120.
840675	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4606 as residues: Pro-134 to Thr-145.
840708	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4607 as residues: Ala-27 to Ser-36.
840848	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4609 as residues: Arg-77 to Asn-82, Glu-119 to Arg-124, Gln-156 to Thr-162, Lys-209 to Lys-215.
840860	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4610 as residues: Ile-27 to Asp-41, Glu-43 to Ala-58, Glu-149 to Glu-154, Lys-158 to Ile-165, Glu-167 to Gly-189, Glu-242 to Phe-247, Arg-259 to Phe-268, Ile-283 to Val-291, Thr-295 to Thr-307, Glu-328 to Asp-338, Asp-372 to Gly-387.
841015	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4611 as residues: Tyr-17 to Thr-29, Lys-35 to Glu-40.
841017	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4612 as residues: Gln-1 to Trp-19.
841030	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4613 as residues: Ser-23 to Gln-30.
841241	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4614 as residues: Asp-41 to Ile-52, Thr-59 to Lys-64, Glu-75 to Asn-89, Thr-99 to Thr-105.
841957	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4615 as residues: Gly-7 to Thr-20, Pro-44 to Thr-49, Gln-55 to Gly-61.
846025	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4616 as residues: Gly-8 to Gly-28, Glu-113 to Asn-122, Arg-144 to Gly-214, Ala-218 to Gly-232, Arg-243 to Glu-248, Glu-356 to Ser-366.

846362	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4617 as residues: His-8 to Gly-18, Phe-66 to Asp-72, Pro-95 to Gly-109, Thr-118 to Ala-126, Gly-128 to Gly-135, Pro-187 to Ser-192, Gly-252 to Arg-258, Asp-270 to Cys-277, Ser-339 to Leu-345, Gly-450 to Ala-468.
846384	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4618 as residues: Gly-3 to Leu-9, Arg-35 to Gly-42, Asp-50 to Thr-55, Ser-98 to Asn-103, Pro-172 to Gly-178, Ser-233 to Pro-243, Ala-289 to Gly-294, Thr-302 to Tyr-309, Glu-341 to Trp-347, Pro-349 to Val-359, Pro-414 to Thr-422, Arg-438 to Glu-443, Gln-507 to Thr-518.
846750	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4619 as residues: Thr-27 to Arg-32, Gly-63 to Gly-71, Ile-95 to Gly-101, Asn-108 to Ser-115.
847598	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4621 as residues: Ser-1 to Thr-27.
848119	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4622 as residues: Pro-5 to Lys-10, Ser-29 to Lys-42, Arg-54 to Arg-66.
848746	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4623 as residues: Pro-61 to Asp-68, Arg-88 to Asp-93.
849084	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4624 as residues: Gly-1 to Pro-8, Ala-48 to Tyr-53, Lys-55 to Arg-62, Glu-67 to Leu-75.
849114	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4625 as residues: Asn-30 to Leu-36, Trp-51 to Phe-56, Pro-62 to Trp-68, Gln-98 to Ser-114, Ile-128 to His-134, Pro-146 to His-151, Asp-153 to Tyr-171, Asp-193 to Trp-198, Pro-222 to Thr-234, Ile-237 to Thr-260, Ile-285 to Gly-296, Arg-301 to Gln-308, Val-311 to Asp-328.
849155	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4627 as residues: Pro-6 to Lys-21, Ala-26 to Val-34, Lys-37 to Ser-46, Phe-73 to Val-81, Pro-86 to Arg-92, Gly-101 to Ser-108, Thr-172 to Pro-178, Met-244 to Lys-255.
849159	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4628 as residues: Thr-28 to Ala-33, Asn-93 to Trp-103, Ile-122 to Pro-130, His-132 to Ile-138.
849244	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4629 as residues: Gln-189 to Glu-196, Glu-206 to Pro-211, Ser-226 to Ile-233, Lys-244 to Ser-253.
849254	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4630 as residues: Ala-5 to Cys-11, Cys-14 to Gly-25, Tyr-32 to Gln-38, Glu-62 to Leu-78, Asp-91 to Tyr-102.
849301	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4631 as residues: Ser-37 to Asp-43, Lys-266 to Ser-272, Glu-304 to Thr-318, Leu-345 to Ser-359, Gln-423 to Ala-439.
849317	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4632 as residues: Pro-42 to Trp-47, Arg-49 to Glu-55, Val-62 to Glu-67, Leu-75 to Leu-90, Leu-102 to Gln-107, Ile-154 to Asp-161.
849332	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4633 as residues: Gln-31 to Ser-38, Gly-60 to Arg-65, Thr-148

	to Thr-155, Cys-180 to Cys-189, Val-224 to Pro-232, Leu-250 to Gln-255.
849422	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4634 as residues: Arg-9 to Arg-14.
849492	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4636 as residues: Ser-5 to Arg-11.
849534	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4637 as residues: Met-8 to His-14.
849565	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4638 as residues: Gly-59 to Ala-67.
849583	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4639 as residues: Pro-13 to Pro-18, Pro-24 to Leu-32, Glu-51 to His-59, Leu-83 to Trp-91, Thr-113 to Gln-120, Pro-133 to Asp-138, Arg-141 to Gln-146, Arg-151 to Ser-156, Tyr-160 to Cys-175, Asn-183 to Asn-188, Trp-221 to Lys-231, Ser-271 to Arg-283, Phe-345 to Gly-350, Ser-381 to Asp-386, Gly-417 to Ser-422, Tyr-462 to Asn-471, Glu-505 to Leu-533, Ser-555 to Asp-561, Thr-566 to His-576, Ser-582 to Gln-587.
849589	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4640 as residues: Ser-16 to Val-25, His-105 to Lys-125, Tyr-147 to Ser-155.
849658	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4641 as residues: Ser-1 to Ser-7.
849666	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4642 as residues: Glu-12 to Met-22.
849679	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4643 as residues: Lys-208 to Asp-214, Glu-278 to Gln-289, Glu-296 to Arg-303, Lys-358 to Leu-364.
849741	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4644 as residues: Arg-30 to His-40.
849783	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4645 as residues: Arg-1 to Pro-14, Gln-47 to Cys-52, Asn-57 to Pro-63, Ser-277 to Lys-282, Leu-326 to Ser-332.
850211	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4646 as residues: Asn-8 to Asn-13.
850254	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4647 as residues: Asn-1 to Arg-6.
850264	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4648 as residues: Ala-33 to Gly-47, Glu-73 to Lys-78, Ser-111 to Asp-126, Gln-139 to Ala-147, Cys-206 to Gly-211, Ser-218 to Asn-225, Leu-237 to Pro-242, Arg-277 to Leu-282, Lys-284 to Lys-291, Ala-357 to Asn-363, Asn-380 to Leu-387, His-475 to Arg-489, Pro-494 to Lys-515.
850273	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4649 as residues: Pro-31 to Lys-38.
850371	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4650 as residues: Lys-32 to Thr-38.
850859	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4651 as residues: Phe-18 to Lys-24, Pro-53 to Lys-75, Tyr-115 to Asp-124, Lys-130 to Leu-137.

851066	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4652 as residues: Pro-6 to Asp-12, Arg-28 to Thr-37, Ile-50 to Lys-59, Ala-63 to Gly-70, Pro-89 to Tyr-96, Ser-103 to Ile-111, Thr-114 to Phe-121, Asp-141 to Pro-147, Arg-162 to Thr-172.
851217	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4653 as residues: Gln-24 to Asp-36, Ser-54 to Thr-65.
852170	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4654 as residues: Leu-13 to Glu-26.
852387	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4655 as residues: Ala-37 to Thr-43.
852812	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4656 as residues: Pro-27 to Pro-33, Asp-92 to Gly-99, Asp-109 to Lys-115, Pro-117 to Trp-130, Phe-208 to Thr-215, Ile-219 to Lys-231, Arg-251 to Asp-257.
853175	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4657 as residues: Gln-21 to Ser-31, Tyr-74 to Gln-81, Leu-115 to Arg-121.
854063	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4659 as residues: Pro-3 to Gly-43.
854073	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4660 as residues: Glu-13 to Val-19, Gln-32 to Met-40, Asp-49 to Arg-54, Leu-74 to Ser-86.
854987	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4661 as residues: Arg-1 to Arg-12.
855130	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4662 as residues: Glu-64 to Tyr-69.
856227	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4663 as residues: Pro-18 to Arg-35, Ala-42 to Gly-54, His-69 to Gln-76, Asp-105 to Arg-110, Arg-121 to Asp-126, Pro-150 to Gln-160.
856243	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4664 as residues: Ala-1 to Ala-8, Lys-78 to Met-86, Arg-126 to Lys-137.
856354	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4665 as residues: Thr-21 to Thr-33.
858178	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4670 as residues: Gly-2 to Gln-8, Lys-68 to Gln-76, Pro-200 to Gly-208, Ser-246 to Gly-257, Gly-280 to Gly-289, Ala-302 to Gly-308, Gly-319 to Asn-331, Leu-352 to Ser-361, Glu-378 to Glu-399, Ala-401 to His-414.
858606	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4671 as residues: Trp-86 to Pro-91.
858894	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4672 as residues: Lys-1 to Ser-9.
858958	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4674 as residues: Pro-19 to Ala-25.
859171	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4675 as residues: Lys-12 to Val-18, Leu-32 to Ser-47, Glu-55 to Asp-66, Glu-94 to Glu-109, Val-115 to Ile-127, Asp-166 to Ser-177, Lys-213 to Glu-225, Glu-241 to Lys-264, Met-322 to Phe-343, Asn-371 to Glu-379, Ala-396 to Ser-407, Ser-415 to Pro-422, Pro-435 to Pro-440,

	Ile-459 to Gln-466, Phe-471 to Phe-476.
859352	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4676 as residues: Thr-11 to Thr-21.
859354	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4677 as residues: Arg-60 to Pro-70, Ser-138 to Ser-145, Cys-157 to Lys-163, Pro-204 to Thr-211, Val-213 to Ser-219, Thr-224 to Thr-230, Pro-297 to Asp-302, Ile-332 to Glu-339, Glu-385 to Ser-390.
859702	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4678 as residues: Lys-7 to Arg-26.
860915	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4679 as residues: Gln-50 to Gly-56.
861209	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4680 as residues: Leu-6 to Thr-15, Pro-85 to Asp-90, Thr-98 to Pro-104.
861534	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4681 as residues: Arg-24 to Ser-30.
861697	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4682 as residues: Gly-8 to Trp-16, Asn-22 to Phe-28, Phe-68 to Arg-75, Ser-93 to Ser-101, Glu-114 to Ile-126, Pro-134 to Phe-143, Gly-165 to Gly-176, Lys-191 to Glu-201, Thr-218 to Lys-227, Tyr-289 to Gln-296.
861826	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4683 as residues: Gly-17 to Pro-23.
861909	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4684 as residues: His-13 to Cys-20, Glu-83 to Cys-93, Pro-131 to Asp-137, Cys-142 to Asn-148, Pro-150 to Gln-155, Pro-160 to Gly-166, Ser-194 to Gly-206, Thr-251 to Ser-258, Gly-267 to Asp-272, Lys-286 to Gly-299, Gln-353 to Leu-366, Thr-368 to Gln-381, Gln-387 to His-397, Glu-404 to Ala-410, Phe-412 to Ala-418, Phe-424 to Ala-439.
862237	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4687 as residues: Cys-20 to Val-27.
862285	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4689 as residues: Ala-26 to Gln-32.
862456	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4691 as residues: Pro-20 to Gly-26, Glu-66 to Trp-76.
862486	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4692 as residues: Cys-36 to Pro-44, His-145 to Asn-151, Asp-186 to Glu-195, Glu-271 to Ile-281, Asp-296 to Pro-302.
863865	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4694 as residues: Gly-1 to Pro-6, Leu-17 to Ala-22, Phe-40 to Ala-45.
863944	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4695 as residues: Glu-102 to Asp-111, Glu-144 to Val-149, Tyr-169 to Lys-180, Arg-239 to Arg-245, Gln-247 to Asp-253, Gly-266 to Asn-278.
864428	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4696 as residues: Thr-1 to Leu-11, Arg-26 to Gly-41, Arg-81 to Asp-91, Asp-144 to Thr-159, Asn-170 to Ala-178, Glu-180 to Lys-191, Cys-249 to Trp-255.
865044	Preferred epitopes include those comprising a sequence shown in SEQ

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	ID NO. 4699 as residues: Thr-17 to Gly-34, Pro-66 to Gly-71, Pro-73 to Val-78.
865421	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4701 as residues: Ala-10 to Glu-16.
866287	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4702 as residues: Val-1 to Leu-6.
866300	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4703 as residues: Thr-28 to Trp-35.
867388	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4707 as residues: Ser-39 to Phe-56, Asp-77 to Arg-84, Glu-103 to Lys-129, Lys-134 to Lys-143, Pro-219 to Gly-227, His-289 to Glu-297, Ala-353 to Arg-360, Pro-409 to Tyr-423, His-433 to Thr-441, Phe-445 to Pro-453, Gln-480 to Leu-488, Pro-526 to Thr-540.
867842	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4708 as residues: Leu-38 to His-44, Leu-46 to Gln-55, Leu-65 to Gln-70, Ile-80 to Arg-88.
867923	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4709 as residues: Leu-17 to Leu-23, Gln-51 to Thr-57.
868035	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4710 as residues: Ser-8 to Pro-13, Pro-21 to Ser-33.
868135	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4711 as residues: Glu-27 to Arg-32, Glu-86 to Gly-93, Ala-117 to Glu-127, Glu-148 to Asn-154, Asp-163 to Ser-174.
868173	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4712 as residues: Thr-6 to Asn-14, Pro-19 to Lys-41.
868224	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4713 as residues: Glu-21 to Glu-31, Arg-37 to Ser-45, Asn-47 to Gly-53, Pro-64 to Arg-70, Ser-97 to Tyr-102, Asp-110 to Val-116.
868655	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4714 as residues: Phe-5 to Ser-21, Ser-24 to Ser-32, Ser-40 to Ser-64, Leu-73 to Glu-81, Pro-122 to Leu-130, Glu-186 to Leu-193, Leu-204 to Trp-213, Ser-278 to Ala-285, Glu-376 to Asp-384, Phe-401 to Val-407.
869698	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4715 as residues: Asp-1 to Ser-6, Glu-16 to Ser-26, Lys-66 to Pro-76, Leu-93 to Arg-99, Val-153 to Lys-164, Glu-177 to Asp-183, Ser-188 to Leu-193, Arg-210 to Ser-220, Thr-229 to Ser-244, Pro-283 to Phe-297.
870190	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4716 as residues: Arg-112 to Lys-118, Gln-168 to His-175.
870349	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4717 as residues: Thr-34 to Ala-39, Ser-42 to Arg-47.
870522	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4719 as residues: Asn-32 to Gly-39, Gly-116 to Lys-124.
870896	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4720 as residues: Leu-21 to Gly-30, Arg-41 to Cys-49, Arg-57 to Phe-62.
871071	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4721 as residues: Arg-1 to Cys-13, Lys-26 to Ile-34.

871225	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4722 as residues: Pro-23 to Gly-36, Arg-77 to Ile-84.
871428	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4723 as residues: Gly-6 to Pro-11.
871498	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4724 as residues: Arg-12 to Ser-18.
871732	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4725 as residues: Ser-56 to Thr-62.
871756	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4726 as residues: Ser-31 to Gly-38.
871821	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4727 as residues: Tyr-25 to Lys-30, Lys-36 to Ile-43, Lys-52 to Gln-69, Glu-76 to Asp-81, Arg-92 to Trp-104, Leu-120 to Lys-126, Ser-129 to Ser-135, Ser-139 to Thr-156, Pro-165 to Glu-178, Ser-181 to Thr-186, Tyr-196 to Lys-201, Cys-225 to Lys-230, Glu-234 to Glu-242.
872354	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4729 as residues: Thr-33 to Lys-43, Lys-81 to Ser-100.
872535	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4730 as residues: Ser-33 to Gly-41, Asn-66 to Asp-73, Cys-136 to Gly-141, Met-187 to Thr-193.
872551	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4731 as residues: Cys-1 to Cys-7, Asp-12 to Arg-27, Pro-49 to Tyr-59, Leu-157 to Leu-163, Ser-243 to Thr-248, Thr-349 to Ser-362, Phe-376 to Ser-385.
872640	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4732 as residues: Tyr-1 to Asp-8, Tyr-33 to Gly-39, Glu-57 to Glu-64, Ser-74 to Val-82, Lys-203 to Arg-214, Gln-229 to Pro-235, Gln-310 to Ala-317, Glu-326 to Asn-331, Gly-366 to Asn-372, Leu-392 to Asn-403, Ala-459 to Gln-466, Asp-494 to His-502, Pro-514 to Leu-522, Glu-614 to Leu-621, Asn-642 to His-651.
872802	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4734 as residues: Ser-1 to Gly-8, Arg-30 to Trp-37.
872852	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4735 as residues: Arg-1 to Gln-7, Arg-22 to Arg-28, Gln-93 to Glu-100.
874307	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4739 as residues: Tyr-1 to Glu-6.
874309	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4741 as residues: Ser-2 to Val-13, Lys-59 to Ser-77.
874310	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4742 as residues: Thr-25 to Thr-31.
874320	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4743 as residues: Ser-1 to Ala-7, Ala-26 to Gly-35, Gly-53 to Phe-59, Arg-67 to Arg-84.
874325	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4744 as residues: Arg-1 to Leu-7, Ser-13 to Val-20, Leu-38 to Glu-44, Leu-79 to Gly-84, Thr-92 to Ala-100, Pro-110 to Ser-119.
874327	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4745 as residues: Asp-45 to Thr-51, Leu-55 to Gly-63, Asp-88 to Phe-97, Gly-185 to Trp-200, Gly-214 to Ser-222, Thr-239 to Val-246.

874329	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4747 as residues: Glu-10 to Ala-16, Asp-32 to His-37.
874348	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4750 as residues: Asn-10 to Thr-15.
874349	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4751 as residues: Pro-1 to Ala-7, Asp-38 to Val-54.
874350	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4752 as residues: Ser-35 to Glu-46, Lys-89 to Asp-94.
874358	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4754 as residues: Phe-34 to Lys-45, Asn-122 to Ser-127, Asp-160 to Lys-165.
874362	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4755 as residues: Ile-1 to Ser-12, His-35 to Glu-47, Glu-55 to Ser-71, Gly-74 to Ser-82, Ala-97 to Ser-139, Lys-153 to Arg-166, Arg-171 to Leu-180, Asp-304 to Gly-309, Glu-373 to Glu-378, Ser-495 to Tyr-500.
874368	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4756 as residues: Ala-14 to Pro-20, Thr-26 to Asn-32, Lys-55 to Ala-61.
874370	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4758 as residues: Arg-48 to Tyr-55, Tyr-64 to Gly-76.
874372	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4759 as residues: Ala-1 to Gly-16, Lys-33 to Thr-44, Leu-52 to Asp-57, Gln-69 to Phe-78, Gly-91 to Cys-104.
874396	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4760 as residues: Leu-39 to Ser-44.
874399	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4761 as residues: Pro-36 to Glu-46, Asn-151 to Asn-170, Tyr-175 to Thr-180, Glu-182 to Glu-190, Thr-202 to Glu-212, Arg-238 to Ser-245, Pro-292 to Gly-302.
874401	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4763 as residues: Gly-10 to Gly-19, Lys-44 to Arg-61, Leu-112 to Lys-117.
874403	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4764 as residues: Phe-20 to Lys-27, Lys-66 to Arg-82.
874413	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4768 as residues: Phe-1 to Asp-11.
874414	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4769 as residues: Ser-54 to Gly-59, Asp-63 to Lys-71.
874416	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4770 as residues: Thr-7 to Ser-14, Pro-28 to Asp-36.
874417	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4771 as residues: Tyr-16 to Ala-26, Ser-43 to Asp-54.
874423	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4773 as residues: Lys-1 to Gly-8, Ser-55 to Leu-60.
874427	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4776 as residues: Tyr-64 to Thr-70.
874435	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4780 as residues: Pro-77 to Lys-95.

874437	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4782 as residues: Glu-15 to Glu-29, Ala-43 to Asp-49, Ile-53 to Asp-65, Lys-86 to Pro-94, Val-102 to Gly-121, Asp-160 to Ser-165, Asn-234 to Lys-241, Glu-309 to Leu-321, Lys-368 to Ala-377, Thr-382 to Asp-400, Ser-407 to Asn-415, Asp-417 to Leu-448.
874438	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4783 as residues: Pro-19 to Leu-28, Pro-44 to Ser-60.
874447	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4784 as residues: Pro-1 to His-6.
874449	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4785 as residues: Glu-10 to Gly-20, Lys-41 to Met-46, Leu-60 to Gln-70.
874455	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4787 as residues: Ile-7 to Lys-15.
874459	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4789 as residues: Tyr-1 to Gly-14, Arg-33 to Pro-41, Pro-58 to Asp-66.
874468	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4793 as residues: Thr-10 to Arg-15.
874469	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4794 as residues: Gln-19 to Lys-26.
874470	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4795 as residues: Arg-3 to Gly-18, Pro-73 to Glu-86, Ser-104 to Pro-117, Gln-143 to Arg-150, Asp-158 to Arg-174, Leu-197 to Ser-222, Ala-235 to Glu-256, Arg-296 to Arg-309.
874473	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4797 as residues: Ser-28 to Arg-37, Arg-83 to Gln-97.
874480	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4801 as residues: Lys-2 to Gly-8, Pro-54 to Asn-65.
874482	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4803 as residues: Lys-52 to Asn-60.
874484	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4804 as residues: Lys-24 to Ser-38.
874486	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4806 as residues: Trp-1 to Pro-10.
874492	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4807 as residues: Arg-33 to Cys-44.
874495	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4808 as residues: Asp-17 to Val-23, Asp-35 to Trp-40, Phe-63 to Arg-68, Ala-150 to Thr-156.
874498	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4809 as residues: Ala-37 to Asn-42, Ala-94 to Glu-106.
874499	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4810 as residues: Met-3 to Pro-10, Pro-18 to Arg-23, Pro-62 to Gly-69.
874503	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4811 as residues: Gln-10 to Glu-21, Ser-28 to Arg-33, Glu-107 to Leu-113, Glu-126 to Ser-133.
874504	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 4812 as residues: Pro-53 to Gly-65, Ala-74 to Lys-96, Lys-107 to Lys-116.
874506	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4814 as residues: Ile-81 to Arg-91.
874518	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4816 as residues: Pro-16 to Ser-24, Thr-34 to Pro-39.
874519	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4817 as residues: Asp-19 to Glu-32, Glu-43 to Glu-80.
874522	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4818 as residues: Pro-6 to Pro-12.
874524	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4819 as residues: Asp-16 to Val-21, Leu-33 to Asp-50.
874527	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4820 as residues: Val-1 to Thr-11, Lys-60 to His-73, Met-84 to Gln-99, Thr-119 to Asp-126.
874528	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4821 as residues: Pro-14 to Arg-23, Ala-171 to Ser-178.
874529	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4822 as residues: Pro-7 to Arg-15.
874545	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4830 as residues: Gly-1 to Asp-6.
874550	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4832 as residues: Arg-20 to Lys-28, Leu-40 to Ala-45, Lys-76 to Ser-81, Leu-106 to Lys-111.
874552	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4834 as residues: Ser-70 to Gly-76.
874553	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4835 as residues: Lys-70 to His-78, Lys-149 to Asn-154, Gly-209 to Leu-217, Lys-248 to Val-255, Ile-259 to Arg-264, Arg-280 to Ala-287.
874556	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4836 as residues: Pro-73 to Ala-78, Ala-95 to Trp-106, Ala-108 to Gly-121, Lys-132 to Asn-142, Glu-163 to Arg-173, Ser-189 to Glu-194, Val-213 to Leu-229, Gln-244 to Asn-260.
874559	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4837 as residues: Thr-47 to Val-63, Arg-90 to Tyr-102, Val-179 to Pro-187, Asp-189 to Gln-200.
874560	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4838 as residues: Arg-222 to Gly-236, Ser-242 to Ile-250, Leu-254 to Ser-260, Glu-277 to Ser-283.
874561	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4839 as residues: Arg-29 to Gln-45.
874562	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4840 as residues: Pro-65 to Val-75, Pro-101 to Ala-131, Pro-143 to Cys-155, Ser-167 to Pro-179, Thr-205 to Cys-216, Arg-218 to His-236, Gln-241 to Asp-267.
874563	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4841 as residues: Ala-1 to Lys-8.
874564	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 4842 as residues: Pro-1 to Cys-8, Glu-48 to His-58, Ser-72 to Glu-78.
874567	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4844 as residues: Met-46 to Leu-55, Leu-93 to Lys-115, Leu-169 to Gly-187, Glu-213 to Gly-219, Lys-224 to Glu-229, Ser-294 to Cys-300, Gln-319 to Leu-328, Ser-345 to Asp-350, Pro-380 to Thr-385, Tyr-387 to Val-393.
874570	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4846 as residues: Pro-3 to Phe-14, Arg-16 to Trp-22, Ser-62 to Leu-74, Asp-86 to Ser-92, Gly-102 to Ser-111, Val-113 to Ser-118.
874571	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4847 as residues: Asp-49 to Asp-59, Asp-110 to Ile-115, Trp-137 to Ser-144.
874573	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4848 as residues: Pro-11 to Ala-35, Phe-47 to Glu-54, Glu-78 to Gly-83, Gln-94 to Ser-106, Ser-114 to Val-120.
874577	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4849 as residues: Leu-1 to Leu-6, Lys-26 to Asp-44, His-50 to Gly-58, Ala-102 to Thr-107.
874580	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4851 as residues: Arg-1 to Val-8, Lys-30 to Tyr-36, Tyr-92 to Gly-101, Lys-116 to Lys-125, Asp-140 to Gly-145, Pro-147 to Ser-167, Ser-170 to Ser-191, Ser-193 to Ile-199, Leu-203 to Arg-215, Ser-220 to Glu-231.
874581	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4852 as residues: Leu-1 to His-8, Pro-74 to Pro-84.
874590	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4855 as residues: Arg-1 to Asn-13, Pro-34 to Pro-41, Val-77 to Thr-84.
874592	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4856 as residues: Val-1 to His-27, Gly-33 to Trp-58, Pro-99 to Cys-105.
874594	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4857 as residues: Lys-18 to Gln-27, Leu-41 to Leu-46.
874601	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4859 as residues: Thr-6 to Gly-14, Gly-20 to Ala-26, Pro-31 to Met-37, Arg-49 to Ser-64, Pro-70 to His-79.
874605	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4861 as residues: Val-5 to Gly-11, Ser-43 to Lys-53, Glu-61 to Thr-68, Thr-99 to Ala-104, Tyr-106 to Asp-120, Asn-139 to Leu-148, Thr-169 to Thr-174, Asn-196 to Asn-202, Asn-223 to Glu-231, Glu-241 to Tyr-247, Ser-265 to Thr-270, Thr-277 to Cys-286, Leu-292 to Asp-298, Asn-347 to Thr-352, Thr-361 to Gly-366, Asn-373 to Thr-383.
874607	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4862 as residues: Pro-1 to Arg-10.
874608	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4863 as residues: Pro-3 to Arg-8, Gly-34 to Thr-53, Asp-60 to Ser-65, Phe-76 to Lys-81.
874609	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4864 as residues: Arg-6 to Arg-13, Phe-25 to Asn-32, Phe-47 to

	Glu-56, Lys-108 to Ala-122.
874610	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4865 as residues: Pro-31 to Trp-39, Pro-101 to Lys-110, Tyr-130 to Ala-137, Val-145 to Lys-154, Pro-174 to Gly-179, Phe-194 to Asn-202, Glu-224 to Gly-240, Thr-259 to Gln-264, Arg-287 to Ser-293, Cys-301 to Gln-307.
874611	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4866 as residues: Lys-1 to Gly-6, Asp-13 to Glu-27.
874615	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4869 as residues: Pro-13 to Cys-19.
874618	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4870 as residues: Arg-10 to Cys-15, Phe-30 to Pro-36, Arg-53 to Ser-59, Thr-66 to Ser-79.
874619	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4871 as residues: Ala-1 to Pro-7.
874621	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4873 as residues: Glu-4 to Gly-12, Thr-21 to Gln-27, Pro-40 to Ser-47, Pro-50 to Ser-61, Val-101 to Cys-107, Lys-138 to Gly-147, Gln-150 to Tyr-156, Lys-169 to Thr-174.
874622	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4874 as residues: Gln-31 to Lys-39, His-55 to Asp-60.
874623	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4875 as residues: Arg-7 to His-24, Pro-27 to Gly-33.
874624	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4876 as residues: Gln-12 to Ser-22.
874626	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4878 as residues: Leu-4 to Gly-11, Pro-60 to Gln-65.
874628	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4879 as residues: Pro-13 to Thr-20, His-24 to Gly-34, Glu-36 to His-42.
874631	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4881 as residues: Lys-14 to Glu-23, Glu-30 to Ser-43, Ser-45 to His-54, Thr-66 to Tyr-71, Pro-75 to Asp-80, Ile-98 to Thr-120, Glu-125 to Lys-133, Leu-146 to Ala-152, Ala-170 to Ile-176, Asp-180 to Cys-200.
874632	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4882 as residues: His-45 to Gly-50.
874635	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4883 as residues: Pro-1 to Pro-7, Leu-19 to Gly-26, Glu-72 to Asp-78, Lys-93 to Glu-103, Gln-152 to Gly-159, Gln-181 to Asp-190, Phe-232 to Val-237, Asn-282 to Thr-287, Pro-289 to Pro-295, His-341 to Asp-351, Cys-378 to Gly-383, Gln-448 to Gly-453, Ser-518 to His-524, Pro-536 to Glu-541.
874636	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4884 as residues: Glu-1 to Tyr-6, Pro-39 to Asp-46.
874639	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4886 as residues: Pro-7 to Gly-29, Ser-36 to Ala-41, Pro-43 to Asp-54, Pro-59 to Leu-64, Gln-70 to Ile-75, Glu-85 to Lys-94.
874642	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4888 as residues: His-8 to Gly-18, Gly-26 to Asp-38.

874644	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4889 as residues: Ser-4 to Leu-10, Thr-25 to Gly-35.
874645	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4890 as residues: Glu-69 to Thr-75.
874650	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4892 as residues: Glu-2 to Glu-14.
874651	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4893 as residues: Arg-1 to His-9.
874652	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4894 as residues: Ser-40 to Asn-45.
874653	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4895 as residues: Thr-1 to Ser-10, Arg-24 to Trp-51, Leu-62 to Gly-67, Pro-72 to Gly-81, Pro-98 to Gly-103.
874655	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4897 as residues: Glu-9 to Cys-14, Ser-38 to Ser-47, Tyr-52 to Lys-61, His-68 to Lys-78, Lys-93 to Gly-101.
874660	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4902 as residues: Leu-13 to Glu-18.
874665	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4904 as residues: Arg-9 to Arg-18, Leu-28 to Phe-36, Pro-49 to Arg-56, His-85 to Asn-103.
874667	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4905 as residues: Leu-47 to Thr-53, Ala-60 to Ser-66.
874670	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4906 as residues: Lys-1 to Leu-6, Pro-9 to Gly-17, Tyr-19 to Glu-25, Arg-30 to Leu-39.
874671	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4907 as residues: Val-5 to Ile-10, Glu-26 to Asp-35, Pro-70 to Pro-80, Tyr-90 to Glu-96.
874673	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4909 as residues: Ser-53 to Ser-63.
874675	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4910 as residues: Ser-33 to Ala-48.
874678	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4911 as residues: Lys-1 to Ser-12.
874679	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4912 as residues: Arg-1 to Glu-7, Leu-21 to Lys-32, His-56 to Cys-64.
874680	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4913 as residues: Glu-8 to Arg-14, Ile-49 to His-59, Leu-86 to Cys-94.
874683	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4915 as residues: Gly-22 to Thr-28, Glu-43 to Val-48, Ser-64 to Leu-71, Phe-106 to Val-111.
874688	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4917 as residues: Ser-10 to Glu-18, Leu-45 to Arg-54.
874689	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4918 as residues: Asn-13 to Gln-19, Lys-56 to Phe-61, Leu-83 to Ala-90.

874695	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4919 as residues: Leu-2 to Ser-12, Pro-125 to Asp-133.
874696	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4920 as residues: Asn-58 to Ser-66.
874699	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4922 as residues: Glu-1 to Ser-7.
874700	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4923 as residues: Gly-10 to Ile-16, Ile-50 to Ser-55.
874701	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4924 as residues: Asn-9 to Gly-14, Glu-17 to His-22.
874702	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4925 as residues: Pro-3 to Arg-20, Pro-24 to Arg-34.
874703	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4926 as residues: Ser-1 to Ser-7, His-35 to Gln-48, Ser-54 to Asn-59, Lys-69 to Met-74.
874708	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4929 as residues: Ala-145 to Gly-152, Val-177 to Gly-185.
874709	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4930 as residues: Ala-13 to Lys-22, Glu-31 to Arg-49, Ser-59 to Asn-65.
874710	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4931 as residues: Glu-1 to Arg-7, Leu-23 to Arg-39, Lys-46 to Asn-52, Pro-59 to Ser-67.
874711	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4932 as residues: Ile-37 to Ala-45, Glu-56 to Pro-62.
874713	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4933 as residues: His-47 to Gly-53, Ser-163 to Ser-169, Pro-276 to Lys-282.
874714	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4934 as residues: Ser-10 to Glu-18.
874715	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4935 as residues: Ser-13 to Leu-18.
874718	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4937 as residues: Gly-43 to His-54, Phe-126 to Cys-132, Pro-140 to Gln-150, Lys-159 to Ala-164, Ser-187 to Gly-193, Pro-212 to Gly-227.
874719	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4938 as residues: Gly-1 to Pro-7, Asp-45 to Asp-50, Lys-82 to Leu-89, Asp-97 to His-102, Thr-118 to Ser-126, Phe-128 to Asp-136, Gly-142 to His-148, Ser-212 to Gln-217, Arg-237 to Glu-244, Arg-269 to Glu-276, Asp-279 to Tyr-284.
874720	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4939 as residues: Glu-18 to Leu-28, Gly-49 to Gly-56, Ser-68 to Arg-74.
874724	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4941 as residues: Asp-7 to Glu-12.
874726	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4943 as residues: Ser-55 to Phe-60.
874732	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 4946 as residues: Val-10 to Gly-15, Ser-98 to Thr-105.
874737	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4947 as residues: Ala-36 to His-45.
874741	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4948 as residues: Gln-11 to His-19, Val-30 to Ile-36, Pro-63 to Ser-69, Gly-78 to Ser-83, Ser-92 to Tyr-97, Gln-155 to Glu-161, Gly-237 to Thr-244.
874744	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4949 as residues: Glu-1 to Phe-12, Ser-47 to Gly-52.
874746	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4951 as residues: Asn-34 to Ser-39.
874749	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4954 as residues: Asp-1 to Gly-17.
874750	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4955 as residues: Gly-4 to Lys-9.
874751	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4956 as residues: His-42 to Glu-47.
874752	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4957 as residues: Ile-11 to Gly-17, Gln-26 to Val-32, Gln-41 to Asp-52.
874756	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4961 as residues: Ser-1 to His-6.
874757	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4962 as residues: Thr-33 to Phe-38.
874760	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4965 as residues: Gly-1 to Ser-8, Ser-23 to Asn-37.
874763	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4966 as residues: Trp-33 to Gln-40, Cys-64 to Ala-70, Ser-148 to Tyr-160.
874764	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4967 as residues: Lys-1 to Gln-19.
874765	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4968 as residues: Thr-50 to Gln-59, Ser-62 to Lys-68.
874766	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4969 as residues: Pro-1 to Gly-21, Leu-37 to Pro-42.
874767	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4970 as residues: Lys-30 to Ala-41, Pro-50 to Asn-56, Glu-141 to Pro-151, Ser-175 to Ser-189.
874769	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4972 as residues: Lys-13 to Glu-22, Glu-76 to Trp-89, Thr-112 to Gly-120, Arg-141 to Gly-146, Thr-178 to Val-185, Val-212 to Arg-223, Pro-225 to Gln-231, Asn-238 to Ala-244, Pro-281 to Glu-287.
874772	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4973 as residues: Gln-44 to Arg-55, Pro-61 to Ala-66.
874774	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4975 as residues: Pro-19 to Pro-34, Leu-46 to Phe-62.
874776	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4977 as residues: Pro-7 to Cys-15, Arg-31 to Glu-42, Ala-47 to Ser-58.

874778	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4978 as residues: Arg-1 to Gly-6.
874779	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4979 as residues: Ser-23 to Glu-31, Asp-46 to Pro-53.
874783	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4982 as residues: Gly-1 to Asp-12, Gly-29 to Gly-37, Gly-73 to Lys-99.
874784	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4983 as residues: Pro-12 to Gly-18.
874785	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4984 as residues: Lys-24 to Lys-36.
874787	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4986 as residues: Thr-5 to Gly-11, Arg-63 to Lys-73, Gln-92 to Glu-98, Ala-106 to Gly-112.
874788	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4987 as residues: Pro-53 to Asn-59.
874790	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4988 as residues: Ser-4 to Thr-9, Gly-17 to Pro-22, Gly-32 to Pro-37.
874791	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4989 as residues: Gly-1 to Ser-6, Pro-20 to Arg-27.
874793	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4990 as residues: Pro-6 to Ala-12, Pro-18 to Thr-28, Pro-31 to Arg-37, Pro-53 to Ile-60.
874795	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4991 as residues: Pro-58 to Leu-72.
874796	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4992 as residues: Thr-4 to Arg-11, Pro-30 to Gly-43, Glu-48 to Glu-56, Met-86 to Ser-92.
874797	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4993 as residues: Gly-52 to Thr-60, Arg-94 to Glu-100.
874800	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4994 as residues: Thr-14 to Tyr-25.
874802	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4996 as residues: Lys-17 to Leu-23.
874803	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 4997 as residues: Glu-7 to Arg-15, Pro-23 to Arg-36, Pro-79 to Ser-96, Ser-119 to Gly-125.
874813	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5004 as residues: Arg-18 to Arg-23, Glu-35 to Asp-50, Ser-67 to Gln-74, Asp-78 to Ser-93.
874815	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5005 as residues: His-38 to Val-46, Ser-97 to Ser-103, Ser-106 to Leu-111, His-191 to Gly-196, Leu-223 to Gly-239, Pro-245 to Ala-250.
874818	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5007 as residues: Tyr-46 to Gly-51.
874819	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5008 as residues: Pro-33 to Gly-40.

874820	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5009 as residues: Ile-18 to Gly-30, Leu-33 to Asn-48.
874821	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5010 as residues: Thr-8 to Ser-16.
874822	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5011 as residues: Asn-9 to Phe-14, Glu-63 to Thr-68.
874827	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5012 as residues: Pro-19 to Ser-24, Val-28 to Glu-34.
874828	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5013 as residues: Lys-17 to Gly-28, Thr-62 to Thr-69, Val-88 to Arg-101, Gln-106 to Pro-112, Arg-127 to Cys-132, Gly-158 to Leu-163.
874830	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5015 as residues: Arg-53 to Thr-58.
874835	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5017 as residues: Gly-1 to Ser-11, Ser-16 to Ala-26, Thr-28 to Ser-36, Gln-53 to Trp-59, Lys-72 to Thr-100, Asp-137 to Cys-143.
874836	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5018 as residues: Leu-12 to Asn-17, Phe-25 to Cys-33, Gln-50 to Ser-60, Glu-63 to Pro-68, Pro-83 to Pro-95.
874837	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5019 as residues: Val-35 to Thr-41.
874844	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5021 as residues: Pro-19 to Phe-26, Pro-29 to Gly-34, Pro-50 to Ser-55, Gly-67 to Lys-73.
874845	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5022 as residues: Asn-1 to Leu-6, Phe-14 to Gly-20.
874847	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5023 as residues: Lys-16 to Thr-22, Glu-36 to Arg-42.
874851	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5025 as residues: Asp-58 to Gly-65, Asp-132 to Cys-147, Pro-149 to Pro-157, Pro-218 to Leu-224.
874852	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5026 as residues: Ala-16 to Trp-21.
874854	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5027 as residues: Gly-2 to Glu-8, Met-21 to Trp-26.
874856	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5029 as residues: His-15 to Asp-20, Lys-27 to Asn-33.
874857	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5030 as residues: Lys-35 to Arg-44, Lys-53 to Val-64, Glu-76 to Val-82, Leu-109 to Lys-118.
874864	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5033 as residues: Leu-40 to Cys-51, Glu-80 to Thr-89, Pro-124 to Ser-132, Cys-153 to Cys-160, Glu-203 to Asp-209, Ala-226 to Arg-241.
874865	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5034 as residues: His-1 to Lys-7.
874871	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5038 as residues: Gly-1 to Ser-10, Ser-13 to Ile-19, Arg-30 to Leu-37, Pro-39 to Asp-48, Pro-140 to Cys-148, Gln-154 to Cys-162,

	Pro-164 to Ser-170.
874873	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5039 as residues: Cys-6 to Ala-12, Pro-14 to Pro-22, Arg-48 to Arg-53, Ile-75 to Thr-85, Glu-97 to Gln-102, Arg-130 to Arg-135, Ser-147 to Val-152, Lys-175 to Thr-185, Phe-189 to Met-194, Gly-213 to Ser-220, Glu-262 to Leu-268.
874879	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5043 as residues: Glu-1 to Gly-15, His-27 to Thr-39, Gly-43 to Ile-49.
874880	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5044 as residues: Pro-62 to Val-70, Lys-103 to Ile-108.
874881	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5045 as residues: Asp-1 to Gly-9.
874885	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5046 as residues: Lys-49 to Gln-55, Glu-83 to Lys-90, Gly-158 to Gly-164, Lys-185 to Gly-192.
874886	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5047 as residues: Pro-10 to Gly-16, His-128 to Gly-134, His-154 to Asp-160, Leu-182 to Leu-187.
874888	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5048 as residues: Pro-15 to Met-27, Thr-106 to His-118, Arg-128 to Arg-139, Val-248 to Arg-254.
874889	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5049 as residues: Pro-7 to Ile-14, Ser-17 to Gln-22.
874890	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5050 as residues: Gly-25 to Ser-31, Trp-34 to Cys-41.
874891	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5051 as residues: Glu-26 to Ser-33, Thr-82 to Phe-90, Met-107 to Asn-114, Thr-125 to Glu-131, His-175 to Asp-180.
874892	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5052 as residues: Arg-1 to Lys-29, Ile-36 to Lys-47, Lys-52 to Gly-83, Pro-89 to Asp-111.
874893	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5053 as residues: Arg-17 to Ile-22.
874896	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5056 as residues: Arg-21 to Lys-26, Pro-37 to Cys-45.
874897	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5057 as residues: Asn-13 to Ala-27, Pro-33 to Lys-42, Asp-61 to Ser-74, Leu-85 to Lys-102.
874898	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5058 as residues: Pro-1 to Leu-9.
874900	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5060 as residues: Lys-3 to Asp-12, Gln-36 to Tyr-47.
874903	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5062 as residues: Pro-9 to Trp-21, Lys-54 to Gln-61, Lys-75 to Phe-87, Glu-97 to Pro-104, Leu-200 to Val-205, Pro-208 to Gly-218, Thr-263 to Leu-278.
874905	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5064 as residues: Tyr-94 to Ile-99.

874906	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5065 as residues: Glu-4 to Pro-11.
874907	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5066 as residues: Gln-1 to Lys-10, Thr-17 to Asn-32, Lys-54 to Lys-65.
874908	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5067 as residues: Ile-1 to Leu-6, Leu-17 to Ala-23, Ile-27 to Thr-33, Asn-40 to Leu-45.
874909	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5068 as residues: Pro-18 to Ser-28, Ser-55 to Thr-64, Asn-90 to Lys-95, Asn-128 to Ile-159, Pro-171 to Gly-178, Pro-186 to Lys-192.
874917	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5071 as residues: Arg-37 to Thr-42, Pro-50 to Gly-68, Pro-70 to Leu-78, Lys-84 to Lys-89, Asn-95 to Val-105, Asp-117 to Lys-126.
874924	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5072 as residues: Leu-8 to Asn-18, Gly-31 to Ala-39.
874925	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5073 as residues: Ser-3 to Arg-9, Gln-24 to Gly-29.
874926	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5074 as residues: Gly-1 to Pro-22.
874928	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5076 as residues: Pro-15 to Gly-23, Ser-27 to Lys-33, Glu-41 to Lys-46, Pro-48 to Asp-55.
874937	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5084 as residues: Ser-15 to Ser-20.
874938	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5085 as residues: Ser-12 to Asp-18, His-43 to Gly-51.
874939	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5086 as residues: Ser-12 to Gln-21.
874946	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5088 as residues: Ser-1 to Lys-6, Lys-16 to Glu-24, Asn-34 to Lys-47.
874957	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5090 as residues: Ala-12 to Asn-20, Pro-23 to Asn-28, Phe-47 to Val-52, Lys-88 to Gly-93, Tyr-113 to Asn-123, Val-211 to Lys-216.
874958	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5091 as residues: Cys-2 to Leu-9, Pro-37 to Gly-42, Ala-50 to Gly-71, Asn-83 to Ala-94, Leu-109 to Leu-115, Phe-156 to Gly-164, Lys-234 to His-249, Glu-267 to Gly-281, Asn-335 to Asp-356, Glu-378 to Ser-385, Gln-402 to Gly-411, Trp-469 to Lys-477, Glu-481 to Gly-486.
874962	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5092 as residues: Asp-1 to Ser-11, Ser-29 to Ser-37, Gln-100 to Arg-112, Leu-123 to Trp-148, Lys-237 to Glu-242, Ala-261 to Asp-266, Asp-279 to Ser-300, Thr-374 to Glu-384, Thr-426 to Thr-432, Glu-443 to Val-449.
874965	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5093 as residues: Asn-13 to His-23, Ser-43 to Gln-56, Val-60 to Glu-65, Pro-67 to Gly-103, Asn-105 to Asp-110.
874970	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 5094 as residues: Pro-3 to Lys-17, Thr-37 to Gly-47.
874972	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5095 as residues: Thr-124 to Thr-129, Gly-136 to Phe-142, Asp-164 to His-171, Asp-180 to Tyr-194.
874973	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5096 as residues: Trp-48 to Arg-56, Pro-68 to Ala-74.
874974	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5097 as residues: Arg-1 to Gly-6, Pro-14 to Ala-26, Ala-42 to Lys-47, Pro-66 to Val-82.
874975	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5098 as residues: Ala-18 to Glu-24, Gln-26 to Gln-31.
874976	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5099 as residues: Lys-13 to Ser-19, Pro-33 to Gly-41.
874981	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5104 as residues: Arg-11 to Arg-20.
874983	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5105 as residues: Lys-1 to Thr-9, Ala-43 to Asp-49, Asp-66 to Arg-72, Gln-80 to Asp-87, Arg-97 to Lys-104, Ser-111 to Glu-117, Phe-150 to Phe-155, Phe-165 to Ala-177, Tyr-219 to Asn-224, Gln-235 to Thr-242, Tyr-244 to Thr-251, Arg-267 to Thr-276, Thr-299 to Ile-306, Pro-318 to Glu-348, Gly-352 to Leu-370.
874984	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5106 as residues: Thr-40 to Glu-46, Lys-51 to Asn-63.
874991	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5110 as residues: Ser-34 to Gln-40, Met-43 to Asp-70.
874993	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5112 as residues: Thr-6 to Gly-12.
874994	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5113 as residues: Val-3 to Lys-9.
874995	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5114 as residues: Arg-1 to Glu-6, Pro-21 to Thr-27, Lys-41 to Thr-48, Gly-202 to Ile-208, Glu-216 to Lys-221, Glu-241 to Lys-247, Glu-261 to Leu-267, Pro-269 to Glu-277, Gln-319 to Lys-326.
874996	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5115 as residues: Glu-1 to Gly-12, Tyr-15 to Pro-22, Asp-36 to Thr-48.
874997	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5116 as residues: Ile-3 to Lys-9, Ser-31 to Trp-40.
874999	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5118 as residues: Lys-11 to Gln-16.
875002	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5120 as residues: Lys-6 to His-16.
875004	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5122 as residues: Pro-5 to Val-14, Asn-24 to Tyr-35, Ser-70 to Val-77, Ser-81 to Asp-99, Ser-121 to Phe-127, Thr-137 to Lys-146, Lys-158 to Ser-164, Phe-185 to Gly-192, Asp-212 to Gln-221.
875005	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5123 as residues: Glu-1 to Ser-14.
875008	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 5125 as residues: Arg-1 to Glu-6, Val-14 to Asp-21.
875009	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5126 as residues: Val-30 to Arg-37, Glu-57 to Thr-63, Leu-66 to Arg-72.
875017	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5128 as residues: Ser-28 to Leu-34, Glu-55 to Gln-62.
875024	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5132 as residues: Tyr-19 to Tyr-24.
875027	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5134 as residues: Thr-46 to Gly-51.
875029	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5135 as residues: Ser-23 to Gly-35.
875034	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5137 as residues: Ser-42 to Trp-53, Glu-71 to Ala-78.
875036	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5139 as residues: Ile-20 to Gly-40.
875037	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5140 as residues: Trp-23 to Gly-28.
875044	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5143 as residues: Gln-23 to Cys-42, Arg-66 to Asn-73.
875045	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5144 as residues: Glu-10 to Leu-25, Lys-27 to Cys-57.
875046	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5145 as residues: Phe-14 to Phe-19.
875049	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5148 as residues: Thr-5 to Lys-12.
875053	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5149 as residues: Ser-16 to Phe-31.
875056	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5151 as residues: Pro-14 to Trp-19.
875058	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5152 as residues: Pro-3 to Gly-20, Gly-24 to Thr-29, Arg-46 to Asn-57, Leu-72 to Phe-78, Glu-81 to Gln-86, Ile-103 to Gln-117, Leu-127 to Ile-142, Asn-144 to Ser-151, Arg-156 to His-166.
875060	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5154 as residues: Pro-14 to Ser-20, Pro-41 to Arg-46, Asp-70 to His-78.
875062	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5156 as residues: Cys-10 to Tyr-16.
875063	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5157 as residues: Ala-18 to Pro-28.
875066	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5158 as residues: Glu-144 to Leu-152, Glu-170 to Asp-179, Gln-225 to Asp-239, Gly-259 to Ala-265.
875067	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5159 as residues: Arg-7 to Pro-16, Pro-37 to Ile-44, Thr-50 to Tyr-72, Pro-88 to Phe-94, Ala-107 to Pro-115.
875068	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5160 as residues: Thr-12 to Trp-23.

875070	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5161 as residues: Asp-17 to Asp-27, Pro-34 to Tyr-40, Glu-52 to Glu-57.
875080	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5163 as residues: Val-30 to Met-37, Glu-39 to Gly-45.
875088	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5165 as residues: Thr-1 to Tyr-8, Gln-27 to Glu-33, Gly-42 to Ser-49, Arg-56 to Lys-81, Cys-97 to Lys-104, His-114 to Ser-133, Gln-139 to Lys-146, Arg-165 to Glu-173, Asp-180 to Lys-188, Arg-196 to Glu-201.
875092	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5166 as residues: Thr-9 to Asp-17, Leu-70 to Lys-95, Asp-115 to Leu-124.
875093	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5167 as residues: Gly-2 to Gly-7, Glu-9 to Gln-16, Cys-24 to Gly-30, Ala-35 to Ala-45, Ala-55 to Ala-60, Cys-79 to Leu-90, Asp-95 to Asp-103.
875094	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5168 as residues: His-80 to Glu-87.
875100	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5170 as residues: Thr-18 to Glu-23.
875102	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5172 as residues: Ser-10 to Gly-16, Pro-24 to Arg-35, Lys-39 to Ala-51.
875103	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5173 as residues: Arg-35 to Ala-41.
875105	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5174 as residues: Phe-70 to His-75.
875106	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5175 as residues: His-45 to Gly-55.
875113	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5177 as residues: Thr-27 to Thr-53.
875114	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5178 as residues: Gly-2 to Arg-7.
875118	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5180 as residues: Pro-21 to Leu-26, Val-62 to Phe-70, Pro-81 to Asp-89.
875121	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5181 as residues: Phe-19 to Leu-36, Glu-38 to Pro-45.
875123	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5182 as residues: Ser-44 to Pro-49, Arg-54 to Gly-64, Leu-94 to Asp-100, Ser-107 to Gly-113, Lys-143 to Tyr-150.
875126	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5185 as residues: His-22 to Ser-27, Cys-34 to Ser-40.
875133	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5187 as residues: His-1 to Gly-9, Gly-19 to Pro-28, Pro-36 to Tyr-42, Gly-44 to Gly-65.
875134	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5188 as residues: Gly-10 to Lys-19, Met-21 to Pro-32.

875143	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5190 as residues: Arg-17 to Ser-23.
875144	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5191 as residues: Asn-14 to Thr-19.
875151	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5193 as residues: Arg-10 to Trp-15, Lys-90 to Ile-95, Asn-103 to Ile-109, Asn-131 to Leu-137, Asn-153 to Arg-163.
875160	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5197 as residues: Val-20 to Asn-27.
875165	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5198 as residues: Thr-5 to Gly-13, Cys-24 to Lys-33.
875177	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5200 as residues: Ala-37 to Asp-44.
875182	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5202 as residues: Pro-25 to Ser-33, Gln-113 to Ser-122, Trp-147 to Tyr-158, Ser-187 to Ala-198, His-201 to Gly-209, Pro-223 to Gly-228, Glu-233 to Gly-238.
875194	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5205 as residues: Ser-16 to Ser-21, Gln-34 to Thr-41.
875200	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5208 as residues: Gln-12 to Cys-19.
875203	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5209 as residues: Arg-1 to Trp-6, Pro-9 to Leu-14.
875205	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5210 as residues: Leu-22 to Ala-27, Ser-31 to Ser-36, Pro-77 to Cys-83.
875206	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5211 as residues: Pro-69 to Pro-75.
875208	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5212 as residues: Asn-25 to Gly-30, Asn-34 to Asn-39.
875209	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5213 as residues: Asn-11 to Ser-18, His-20 to Arg-26, Val-31 to Trp-41.
875210	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5214 as residues: Leu-37 to Thr-52.
875214	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5216 as residues: Ala-7 to Leu-33.
875215	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5217 as residues: Gln-18 to Leu-29, Asp-52 to Ile-57.
875223	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5218 as residues: Thr-2 to Gln-7.
875226	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5219 as residues: Arg-1 to Gln-7, Lys-21 to Gln-31, Leu-41 to Ser-84, Asp-87 to Arg-98, Leu-102 to Lys-115, Leu-129 to Lys-139.
875228	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5220 as residues: Ser-1 to His-10, Pro-84 to Arg-98, His-108 to Asn-113.
875240	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5224 as residues: Ser-31 to Arg-43.

875246	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5225 as residues: Phe-29 to Leu-37.
875261	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5228 as residues: Ser-10 to Asp-24.
875270	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5230 as residues: Ser-1 to Ser-11, Gln-64 to Gln-69, Arg-117 to Pro-128, Pro-135 to Asp-140, Gly-147 to Arg-160, Lys-168 to Val-173, Asn-181 to Lys-191, Glu-200 to Gly-205, Gly-215 to Lys-224.
875271	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5231 as residues: Phe-12 to Lys-17.
875275	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5232 as residues: Pro-9 to Gly-20.
875277	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5234 as residues: Arg-6 to Ser-18.
875278	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5235 as residues: Thr-45 to Lys-50.
875282	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5239 as residues: Thr-14 to Lys-31.
875287	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5240 as residues: Lys-15 to Trp-31, Val-44 to Cys-51.
875288	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5241 as residues: Pro-28 to Gly-39, Ser-42 to Ser-50, Arg-61 to Arg-70, Gln-75 to Gly-86.
875296	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5243 as residues: Glu-26 to Ala-32, Thr-81 to Ser-90.
875303	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5244 as residues: Glu-2 to Met-9, Asp-17 to Asn-22, Leu-27 to Val-35.
875306	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5247 as residues: Thr-17 to Phe-22.
875307	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5248 as residues: Pro-1 to Tyr-22.
875308	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5249 as residues: Pro-36 to Pro-41.
875309	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5250 as residues: Pro-1 to Ala-9, Gly-42 to Gln-51.
875312	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5253 as residues: Leu-7 to Tyr-14, Glu-41 to Leu-49.
875313	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5254 as residues: Gln-23 to Leu-34, Asp-45 to Arg-60.
875316	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5255 as residues: Asn-25 to Tyr-31.
875319	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5256 as residues: Asp-10 to Lys-16, Lys-35 to Asn-41, Tyr-55 to Leu-62, Glu-145 to Thr-153, Ser-169 to Lys-175, Thr-184 to His-192, Gly-224 to Trp-234, Ala-251 to Leu-256, Glu-276 to Asp-281.
875336	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5261 as residues: Tyr-3 to Leu-10.
875338	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 5262 as residues: Pro-9 to Ile-14, Glu-81 to Gln-90.
875346	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5264 as residues: Gly-29 to Arg-44.
875347	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5265 as residues: Ile-3 to Ser-14, Ala-32 to Ser-44, Ser-60 to Leu-67.
875360	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5268 as residues: Pro-14 to Leu-19, Ile-37 to Ala-46, Ser-58 to Asn-65, Pro-71 to Gly-77.
875364	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5269 as residues: Val-38 to Phe-47, Asn-64 to Phe-69.
875367	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5271 as residues: Gly-14 to Leu-21, Asn-31 to Met-37.
875371	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5272 as residues: Pro-12 to Glu-23, Lys-29 to Pro-34, Pro-54 to Leu-66.
875372	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5273 as residues: Ala-7 to Arg-12.
875373	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5274 as residues: Tyr-54 to Cys-61, Asn-73 to Pro-78, Pro-84 to Asn-93, Gln-99 to Asp-105.
875378	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5276 as residues: Leu-42 to Lys-53, Cys-100 to Asn-110, Pro-137 to Gly-144, Pro-190 to Ala-205.
875379	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5277 as residues: Asp-5 to Ala-10, Ala-19 to Ile-25, Val-39 to Ser-44, Gln-74 to Cys-90, Leu-94 to Glu-99, Leu-108 to Phe-116.
875381	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5279 as residues: Cys-46 to Leu-51.
875382	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5280 as residues: Pro-11 to Thr-16, Pro-23 to Gly-33, Ala-51 to Arg-61.
875384	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5281 as residues: Gln-15 to Gly-28, Asp-83 to Tyr-92.
875385	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5282 as residues: Leu-3 to Asp-8, Gln-30 to His-36.
875388	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5283 as residues: Thr-2 to Ser-9, Pro-23 to Arg-30.
875391	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5284 as residues: Lys-1 to Arg-10, Lys-53 to Tyr-62.
875397	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5285 as residues: Arg-7 to Gly-29, Arg-37 to Glu-47, Asp-78 to Thr-83, Gly-173 to Val-180, Glu-188 to Glu-202, Pro-208 to Thr-216, Thr-227 to Glu-242, Arg-250 to Gly-281, Lys-288 to Thr-296, Glu-301 to Arg-311, Ala-313 to Lys-318, Lys-357 to Thr-367, Pro-376 to Ser-387, Pro-416 to Lys-428, Pro-486 to Thr-491, Ser-497 to Arg-516, Lys-522 to Lys-532, Arg-537 to Met-557.
875402	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5286 as residues: Asn-1 to Thr-15.

875406	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5288 as residues: Pro-5 to Ala-19.
875410	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5289 as residues: Ala-4 to Pro-14, Pro-23 to Thr-28, Thr-40 to Gln-45, Tyr-60 to Gln-69, Pro-88 to Leu-93, Glu-108 to Ala-113, Val-119 to Gly-131, Arg-146 to Arg-155, Ala-164 to Lys-171, Thr-190 to Met-201.
875415	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5290 as residues: Arg-18 to Trp-23, Gly-25 to Gly-32, Lys-34 to Arg-42, Gly-52 to Thr-59, Ala-86 to Lys-92.
875416	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5291 as residues: Lys-9 to Gly-37.
875417	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5292 as residues: Glu-2 to Cys-14.
875419	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5294 as residues: Thr-2 to Tyr-11.
875423	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5295 as residues: Lys-13 to Ile-24, Phe-28 to Val-35.
875428	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5298 as residues: Gly-2 to Thr-7, Gly-20 to Thr-29, Asn-69 to Arg-77.
875429	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5299 as residues: Phe-4 to Pro-9, Pro-13 to Gln-18.
875433	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5300 as residues: Lys-78 to Met-83.
875434	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5301 as residues: Thr-34 to Glu-39.
875437	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5302 as residues: Glu-1 to Gln-7.
875440	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5303 as residues: Arg-11 to Met-17, Ile-66 to Trp-71, Asp-91 to Leu-97, Ala-102 to Lys-111, Trp-113 to Glu-120, Pro-132 to Asn-141, Thr-144 to Glu-153, Glu-159 to Glu-172, Pro-177 to Lys-192.
875441	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5304 as residues: Cys-28 to Cys-34.
875442	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5305 as residues: Pro-18 to Lys-23.
875446	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5306 as residues: Pro-8 to Phe-18.
875452	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5307 as residues: Ala-6 to Cys-17.
875458	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5308 as residues: Glu-40 to Glu-46, Arg-51 to Ser-67.
875462	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5311 as residues: Ser-2 to Ser-14, Arg-75 to Asn-85.
875468	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5313 as residues: Thr-35 to Thr-49.
875474	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5314 as residues: Asp-1 to Asp-13, Arg-40 to Arg-56, Ser-72 to

	Asp-84, Ala-88 to Arg-96, Lys-115 to Phe-121, Asp-133 to Lys-139, Leu-203 to Leu-210, Asp-264 to Arg-269.
875475	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5315 as residues: Pro-12 to Gly-19.
875479	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5318 as residues: His-32 to Lys-40.
875481	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5319 as residues: Arg-22 to Ser-39, Val-42 to Thr-54, Gln-61 to His-69, Glu-83 to Gly-109, Pro-111 to Gly-118.
875490	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5322 as residues: Cys-75 to Thr-81.
875491	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5323 as residues: Gln-8 to His-15, Ser-32 to Gln-43, Leu-51 to Glu-70.
875499	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5329 as residues: Asn-36 to Leu-55.
875500	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5330 as residues: Thr-31 to Arg-39.
875501	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5331 as residues: Asp-52 to Asn-59.
875508	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5334 as residues: Pro-1 to Ile-18, Asp-28 to Lys-33, Leu-50 to Gln-55, Glu-85 to Ala-94, Leu-121 to Ser-130, Lys-143 to Gly-150, Leu-173 to Asp-179, Lys-183 to Asp-192, Lys-196 to Glu-202, Asn-219 to Asn-227, Glu-235 to Glu-248.
875512	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5335 as residues: Asp-10 to Trp-16, Glu-33 to Asn-43.
875514	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5336 as residues: Asp-11 to Tyr-32, Gln-43 to Thr-58, His-70 to Arg-79, Ser-101 to Ala-108, Met-110 to Ser-124.
875515	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5337 as residues: Met-1 to Arg-8, Met-10 to His-17.
875516	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5338 as residues: Leu-2 to Ser-8, Gln-41 to Gly-46, Asp-70 to Gln-80, Pro-82 to Gly-97.
875518	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5340 as residues: Arg-1 to Trp-11, Ser-28 to Leu-42, Gly-65 to Gly-70, Ala-72 to Gln-77, Gly-89 to Lys-98, Asp-126 to Thr-136, Gln-218 to Gly-226, Lys-261 to Gly-282.
875520	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5341 as residues: Arg-5 to Ser-18, Arg-36 to Gly-42, Gln-45 to Gly-56, Val-69 to Arg-75.
875525	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5343 as residues: Arg-6 to Thr-22, Arg-31 to His-38.
875527	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5344 as residues: Gly-24 to Leu-31, Ser-64 to Val-70, Arg-93 to Trp-100.
875528	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5345 as residues: Thr-6 to Ile-13.

875534	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5347 as residues: Arg-1 to Thr-14, Arg-28 to Asp-34, Gln-51 to Ser-60, Lys-69 to Gly-78, Val-110 to Val-115, Asn-135 to Glu-141, Asn-167 to Pro-179, Lys-203 to Lys-214, Gly-267 to Pro-279.
875538	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5348 as residues: Thr-1 to Arg-6.
875544	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5351 as residues: Gln-1 to Asn-8.
875545	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5352 as residues: Cys-2 to Gly-16, Glu-35 to Leu-40, Pro-61 to Gln-66.
875547	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5354 as residues: Leu-37 to His-43.
875548	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5355 as residues: Val-15 to Asp-21, Cys-29 to Ser-36.
875550	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5356 as residues: Arg-81 to Gln-93, Leu-103 to Val-116.
875551	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5357 as residues: Glu-11 to Lys-22, Glu-36 to Gly-41.
875553	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5359 as residues: Arg-6 to Lys-11, Phe-16 to Ile-21, Thr-48 to Leu-56, Pro-64 to Arg-70.
875554	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5360 as residues: Tyr-2 to Ser-10, Asn-69 to Leu-80.
875559	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5363 as residues: Pro-123 to Asn-130.
875563	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5365 as residues: Pro-35 to Gly-62.
875565	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5367 as residues: Pro-2 to Asp-7, Gln-13 to Gln-29, Pro-35 to Trp-41.
875570	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5369 as residues: Leu-1 to Ser-6, Ser-45 to Lys-56, Asn-66 to Lys-78.
875574	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5372 as residues: Pro-10 to Gln-15, Cys-25 to Ile-30, Ser-42 to Lys-47.
875583	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5374 as residues: Lys-6 to Lys-37, Arg-43 to Leu-49, Met-53 to Val-59.
875590	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5380 as residues: Cys-128 to Pro-134.
875594	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5381 as residues: Gly-40 to Ser-45.
875596	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5382 as residues: Gly-1 to Gly-10.
875597	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5383 as residues: His-3 to Ser-9.
875604	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 5386 as residues: Lys-7 to Ser-20, Arg-67 to Ser-74.
875605	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5387 as residues: Gly-17 to Ser-24, Met-42 to Arg-48.
875606	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5388 as residues: Tyr-1 to Gly-13, Glu-32 to Asp-43, Ser-55 to Ile-62, Pro-119 to Asn-131.
875609	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5390 as residues: Thr-12 to Ser-20, Leu-60 to Ala-66.
875610	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5391 as residues: Cys-41 to Ser-47.
875613	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5394 as residues: Leu-12 to Lys-18.
875625	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5395 as residues: Asp-8 to Leu-25, Arg-94 to Ala-102, Glu-133 to Ala-138.
875628	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5396 as residues: Ser-17 to Gly-23.
875629	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5397 as residues: Glu-1 to Glu-11, Arg-21 to Ser-27.
875631	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5399 as residues: Val-37 to Asn-43, Glu-62 to Pro-69, Gln-118 to Tyr-131, Ser-144 to Trp-150.
875633	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5401 as residues: Asn-11 to Arg-16.
875634	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5402 as residues: Ile-1 to Gly-10, Asp-24 to Arg-29.
875635	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5403 as residues: Phe-1 to Ile-8, Thr-21 to Leu-38, Glu-55 to Lys-70, Lys-76 to Leu-82, Lys-84 to Glu-89, Ile-93 to Ser-98.
875636	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5404 as residues: Pro-30 to Asp-35.
875638	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5405 as residues: Asp-1 to Gly-7, Arg-13 to Arg-18, Arg-48 to Ser-54.
875640	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5407 as residues: Thr-36 to Cys-47.
875642	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5409 as residues: Arg-2 to Thr-8, Thr-46 to His-51.
875646	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5410 as residues: Ala-4 to Arg-10, Cys-22 to Lys-27.
875650	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5411 as residues: Glu-29 to Lys-34, Leu-151 to Tyr-156, Glu-162 to Arg-170.
875651	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5412 as residues: Leu-119 to Gln-125, Arg-128 to Ser-139, Gln-145 to Pro-158.
875653	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5413 as residues: Pro-1 to Gln-14.
875654	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 5414 as residues: Arg-34 to Gly-66.
875658	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5415 as residues: His-19 to Tyr-30, Ala-53 to Ala-59, Ala-90 to Pro-101, Lys-132 to Lys-139, Ala-152 to Arg-158, Phe-168 to Leu-175, Arg-178 to Lys-186.
875661	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5416 as residues: Tyr-2 to Ser-8, Thr-15 to Ala-25.
875662	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5417 as residues: Gly-5 to Cys-12, Phe-40 to Thr-47.
875663	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5418 as residues: Thr-4 to Ser-12.
875665	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5419 as residues: Lys-2 to Lys-7.
875669	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5420 as residues: Lys-1 to Gly-11.
875677	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5423 as residues: Gly-1 to His-7, Val-10 to Phe-17, Asp-62 to Arg-67.
875678	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5424 as residues: Ile-2 to Ile-9, Asn-76 to Gln-82.
875681	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5426 as residues: Glu-1 to Asn-12, Pro-20 to Ala-26, Thr-42 to Ser-50.
875683	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5428 as residues: Val-60 to Pro-69.
875687	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5429 as residues: Asp-18 to Phe-24.
875688	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5430 as residues: Glu-8 to Glu-13.
875689	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5431 as residues: Lys-24 to Lys-30.
875690	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5432 as residues: Gly-3 to Leu-20, Trp-38 to Arg-44, Lys-58 to Lys-64.
875698	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5434 as residues: Tyr-43 to Lys-52, Glu-60 to Arg-66, Gln-84 to Cys-89, Gln-106 to Lys-117, Thr-140 to Asp-168, Gln-170 to Arg-177.
875704	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5438 as residues: Gly-24 to Thr-30, Ser-103 to Gly-109.
875717	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5441 as residues: Cys-12 to Cys-34, Pro-36 to Thr-45, Arg-75 to Asn-85.
875719	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5442 as residues: Asn-1 to Tyr-7.
875722	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5443 as residues: Leu-2 to Phe-7.
875724	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5444 as residues: Asn-86 to Ser-91.

875725	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5445 as residues: Thr-9 to Thr-17, Arg-33 to Val-41.
875727	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5446 as residues: Thr-16 to Pro-23, Pro-39 to Trp-48, Arg-50 to Lys-55, Gly-73 to Gly-79.
875734	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5451 as residues: Ser-12 to Thr-18, Pro-20 to Pro-25.
875736	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5452 as residues: Phe-10 to Arg-15, Ile-48 to Thr-53, Ser-64 to Asn-69.
875737	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5453 as residues: Leu-1 to Cys-6, Ala-74 to Gly-87, Gln-106 to Gly-111.
875738	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5454 as residues: Glu-11 to Asp-19, Gly-40 to Thr-47, Pro-66 to Arg-71.
875739	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5455 as residues: Gly-45 to Asp-50.
875740	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5456 as residues: Glu-1 to Gln-22.
875746	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5457 as residues: Leu-55 to Gln-64.
875751	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5459 as residues: Phe-21 to Leu-26, Gly-81 to His-87.
875752	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5460 as residues: Ser-11 to Asn-16, Trp-33 to Arg-49.
875753	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5461 as residues: Glu-1 to Ile-17, Leu-54 to Asn-59.
875754	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5462 as residues: Arg-53 to Val-58.
875760	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5463 as residues: Phe-45 to Asn-51.
875765	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5465 as residues: Pro-7 to Gly-12.
875766	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5466 as residues: Gly-21 to Phe-28.
875769	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5468 as residues: Lys-7 to Gly-12.
875772	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5469 as residues: Arg-19 to Pro-45, Gly-60 to Leu-72, Leu-90 to Asn-109.
875774	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5471 as residues: Ile-27 to Val-33, Val-63 to Ser-68.
875779	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5473 as residues: Gln-54 to Ser-63, Glu-84 to Lys-92, Val-100 to Gln-105.
875781	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5475 as residues: Glu-72 to Ala-80.
875783	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 5477 as residues: Gly-1 to Asn-15.
875784	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5478 as residues: Glu-17 to Asp-22, Asn-30 to Cys-35, Leu-39 to Lys-49.
875786	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5480 as residues: Arg-8 to Thr-17.
875787	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5481 as residues: Ser-3 to Pro-16, Asp-38 to Ser-43, Arg-53 to Gln-62, Trp-78 to Ser-84.
875789	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5482 as residues: Arg-1 to Ile-8, Pro-50 to Thr-62.
875794	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5484 as residues: Thr-8 to Val-13, Tyr-39 to Arg-46.
875800	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5486 as residues: Tyr-1 to Gln-12, Gly-17 to Cys-26, Trp-37 to Asn-43, Leu-46 to Gly-51.
875804	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5488 as residues: Asp-54 to Gly-67.
875805	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5489 as residues: Ser-1 to Thr-9.
875809	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5491 as residues: Asn-16 to Leu-30, Ala-48 to Thr-53, Arg-109 to Asp-114, Arg-120 to Gly-126, Pro-153 to Asp-161, Asn-177 to Lys-186, Ser-253 to Ser-260.
875810	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5492 as residues: Pro-1 to Lys-11, Pro-31 to Leu-39, Thr-67 to Lys-77.
875814	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5493 as residues: His-1 to Gly-14, Ala-21 to Arg-30.
875815	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5494 as residues: Ile-14 to Leu-35, Pro-37 to Thr-51.
875817	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5496 as residues: Ser-15 to Ile-24, Asn-56 to Lys-67, Ser-80 to Lys-95, Gly-148 to Pro-165.
875820	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5498 as residues: Phe-2 to Ser-9, Cys-12 to Ser-23, Glu-37 to Pro-48, Glu-56 to Asp-64.
875821	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5499 as residues: Gly-98 to Ala-110.
875822	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5500 as residues: Ala-7 to Pro-18, Ser-57 to Ser-64, Phe-94 to Gln-105, Leu-129 to Gly-141.
875825	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5502 as residues: Lys-1 to Lys-19, Glu-66 to Gln-73, Asn-75 to Asn-80, Met-112 to Asn-118, Val-122 to Thr-134.
875828	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5504 as residues: His-1 to Leu-12, Leu-16 to Cys-30, Thr-46 to Asn-56.
875832	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 5505 as residues: Lys-1 to Arg-9, Cys-32 to Tyr-39, Lys-53 to Gly-64, Phe-86 to Asn-92.
875836	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5508 as residues: His-79 to Ser-92.
875837	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5509 as residues: Ser-47 to Arg-54.
875838	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5510 as residues: Ser-1 to Phe-8.
875839	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5511 as residues: Gln-1 to Gly-22, Pro-36 to Arg-42, Arg-89 to Gln-94.
875840	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5512 as residues: Thr-6 to Asn-16, Gln-50 to Lys-66.
875841	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5513 as residues: Ala-44 to Arg-51, Val-71 to Ser-76.
875845	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5514 as residues: Gly-1 to Lys-6, Ser-54 to Ser-60.
875846	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5515 as residues: Ser-28 to Gly-33.
875855	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5521 as residues: Glu-13 to Asn-18, Asn-53 to Lys-59.
875856	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5522 as residues: Ala-28 to Ser-33.
875858	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5523 as residues: His-1 to Asn-17, Gly-21 to Arg-28, Lys-43 to Asn-49, Ser-64 to His-80, Ala-91 to Asp-130, Gly-144 to Ser-158.
875863	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5524 as residues: Pro-23 to Asp-28, Pro-40 to Gln-47.
875864	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5525 as residues: Pro-1 to Ser-15, Leu-27 to Lys-32, Arg-39 to Ser-53, Thr-58 to Glu-81, Gly-87 to Leu-92, Val-96 to Glu-106, Lys-114 to Ile-131, Asp-134 to Lys-140, Asn-142 to Lys-149, Lys-155 to Gly-168.
875865	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5526 as residues: His-11 to Cys-23, Ala-29 to Gln-35, His-43 to Arg-50.
875868	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5527 as residues: Arg-33 to Glu-42, Arg-45 to Gly-64, Ala-79 to Asn-117, Thr-130 to Lys-143, Ser-222 to Lys-233, Val-235 to Asn-240, Leu-289 to Met-294.
875871	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5528 as residues: Gln-1 to Ala-17, Gln-43 to Asp-48.
875874	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5529 as residues: Glu-40 to Thr-50.
875884	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5530 as residues: Ser-14 to Cys-19, Lys-53 to Asn-58, Ser-63 to Ser-70, Gly-118 to Cys-123, Cys-132 to Gly-138.
875886	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5531 as residues: Asn-46 to Glu-51.

875888	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5532 as residues: Lys-1 to Gly-17, Arg-56 to Gln-61, Gln-82 to Pro-89.
875891	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5533 as residues: Tyr-4 to Gly-11, Phe-33 to Asn-38.
875894	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5534 as residues: Arg-11 to Glu-24, Arg-39 to Glu-52, His-70 to Gly-82, His-98 to Arg-124, His-126 to Ser-142, His-154 to Gly-166.
875897	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5535 as residues: Pro-1 to Lys-8, Phe-49 to Pro-67, Leu-88 to Trp-100.
875905	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5539 as residues: Pro-19 to Cys-28, Leu-40 to Thr-49, Glu-57 to Pro-69, Phe-82 to Asn-89.
875908	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5542 as residues: Val-27 to Gly-34.
875912	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5543 as residues: Lys-5 to Gln-11, Ser-16 to Lys-28, Pro-39 to Phe-44, Thr-136 to Lys-148, Cys-182 to His-189, Val-197 to Tyr-202, Ser-273 to Gly-300.
875914	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5545 as residues: Ser-7 to Lys-13, Met-16 to Trp-21, Pro-54 to Gly-60, Ser-112 to Gly-117.
875923	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5547 as residues: Asn-1 to Lys-10, Glu-29 to Thr-35, Glu-41 to Glu-57, Glu-78 to Arg-83, Ala-97 to Trp-102.
875924	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5548 as residues: Gln-1 to Asn-8, Arg-22 to Leu-28, Ser-30 to Phe-48, Ser-51 to Glu-56, Gln-70 to Leu-88, Phe-101 to Asn-111, Arg-113 to Tyr-121, Ser-130 to Asn-135, Glu-141 to Gln-152, Asn-169 to Trp-191.
875925	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5549 as residues: Ser-45 to Ala-50.
875926	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5550 as residues: Leu-4 to Ser-13.
875927	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5551 as residues: Arg-2 to Lys-21.
875932	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5552 as residues: Asp-27 to Gln-33.
875933	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5553 as residues: Gly-1 to Gln-8, Met-19 to Ser-24.
875935	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5555 as residues: Asn-20 to Thr-25, Ser-30 to Pro-35.
875936	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5556 as residues: Gly-12 to Lys-18, Arg-46 to Glu-56, Leu-67 to Gly-73, Ala-91 to Ser-102.
875937	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5557 as residues: Arg-4 to Thr-10, Arg-61 to Glu-71, Leu-82 to Gly-88, Ala-106 to Lys-142.

875939	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5559 as residues: Arg-3 to Leu-15, Arg-17 to Asn-24.
875940	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5560 as residues: Gly-28 to Phe-34, Gly-36 to Cys-41, Arg-46 to Arg-54, Pro-75 to Arg-90.
875941	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5561 as residues: Gln-24 to Glu-35, Lys-53 to Gln-67, Pro-85 to Trp-98.
875942	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5562 as residues: Cys-74 to Ala-84.
875946	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5563 as residues: Gly-34 to Pro-48, Arg-86 to Gly-91.
875951	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5565 as residues: Pro-31 to Leu-41.
875955	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5568 as residues: His-19 to Asn-24, Pro-39 to Lys-45.
875967	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5569 as residues: Arg-30 to Arg-38.
875971	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5570 as residues: Ser-1 to Asp-8, Asn-16 to Ser-35, Asn-47 to Pro-70.
875972	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5571 as residues: Pro-14 to Arg-23, Phe-41 to Gly-49, His-69 to His-76, Tyr-84 to Asn-90.
875976	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5573 as residues: Tyr-3 to Gly-10.
875984	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5576 as residues: Ser-2 to Gln-15.
875991	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5579 as residues: Thr-47 to Gly-53.
875995	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5581 as residues: Pro-3 to Glu-8.
875999	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5584 as residues: Gly-11 to Ala-16, Gln-70 to His-78.
876006	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5586 as residues: Pro-12 to Thr-22.
876008	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5588 as residues: Cys-2 to Asn-10.
876012	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5590 as residues: Trp-30 to Thr-43.
876018	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5592 as residues: Pro-52 to Asn-63, Pro-70 to Ile-79, Arg-93 to Gln-111.
876021	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5594 as residues: Ala-59 to Ser-72, Ser-84 to Leu-94, Thr-98 to Lys-105, Val-109 to Glu-119, Asn-124 to Leu-139, Pro-146 to Ala-155, Ser-161 to Thr-190, Glu-216 to His-221, Asn-229 to Gly-240, Ile-258 to Gly-269, Thr-300 to Thr-310, Thr-312 to Ser-317.
876022	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 5595 as residues: Leu-2 to Tyr-11, Glu-55 to Thr-60.
876023	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5596 as residues: Lys-45 to Phe-58, Pro-99 to Gly-105, Arg-124 to Arg-130.
876024	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5597 as residues: Cys-7 to Arg-12, Pro-32 to Ser-49, Arg-59 to Gly-70, Ala-74 to Arg-82.
876028	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5601 as residues: Gly-46 to Gly-51.
876029	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5602 as residues: Ala-4 to Thr-9, Gln-17 to Thr-40.
876044	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5606 as residues: Asn-6 to Lys-12, His-32 to Phe-41.
876045	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5607 as residues: Thr-5 to Glu-14, Pro-23 to Tyr-28, Arg-42 to Pro-49, Lys-87 to Ser-95.
876048	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5608 as residues: Gln-1 to Asp-11, Arg-18 to Gly-23, Thr-31 to Pro-38.
876057	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5611 as residues: Glu-17 to Ser-42.
876059	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5612 as residues: Pro-34 to His-49.
876065	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5614 as residues: Ser-28 to Val-33, Gln-41 to Gln-46, Gln-53 to Gln-63, Ala-76 to His-84, Ile-88 to Ser-93, Pro-99 to Ala-105, Pro-114 to Ser-122, Pro-145 to Thr-153, Pro-197 to Gln-206.
876078	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5616 as residues: Arg-71 to Trp-80, Arg-88 to Arg-99.
876079	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5617 as residues: Cys-16 to His-21, Lys-23 to Asp-31.
876081	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5618 as residues: Pro-6 to Cys-12.
876086	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5620 as residues: Cys-66 to Ser-74, Arg-81 to His-90.
876089	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5622 as residues: Ser-2 to Gly-11.
876090	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5623 as residues: Gln-1 to Glu-13, Lys-25 to Ser-34, Asp-49 to Gln-54.
876091	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5624 as residues: Phe-14 to Tyr-19, Arg-24 to Arg-32.
876093	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5625 as residues: Ser-1 to Glu-8, Asp-30 to Gly-37, Val-44 to Glu-58.
876094	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5626 as residues: Gly-1 to Gly-7, Ile-23 to Ala-29, Phe-40 to Gln-45.
876095	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 5627 as residues: Lys-1 to Lys-6, Pro-8 to Glu-19.
876097	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5628 as residues: Arg-30 to Ser-37.
876098	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5629 as residues: Leu-18 to Leu-23.
876101	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5630 as residues: Gly-56 to Asp-62.
876104	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5631 as residues: Gln-1 to Glu-7, Ala-31 to Glu-48.
876107	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5633 as residues: Gly-13 to Gln-19, Arg-58 to Gly-63, Leu-129 to Pro-134.
876118	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5637 as residues: Pro-35 to Gly-42, Pro-62 to Arg-74, Val-87 to Ala-93, Leu-119 to Ala-124.
876121	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5638 as residues: Pro-2 to Pro-35, Ser-40 to Leu-57, Thr-83 to Thr-93, His-96 to Thr-105, Leu-114 to Gly-125, Asp-128 to Asp-133, Lys-146 to Ser-156.
876140	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5644 as residues: Ala-39 to Leu-47, Val-49 to Lys-55, Thr-66 to Asp-75, Thr-85 to Gly-104, Ala-114 to Gly-147, Pro-176 to Thr-199, Ser-205 to Ser-221, Glu-233 to Lys-240, Lys-246 to Asp-251, Glu-256 to Ser-267, Ser-291 to Leu-302, Thr-305 to Asp-324, Cys-336 to Val-345, Phe-367 to Cys-375.
876151	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5648 as residues: Gly-101 to Arg-106.
876152	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5649 as residues: Arg-1 to Gly-12, His-33 to Leu-42.
876155	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5651 as residues: Phe-26 to Lys-51, Gln-61 to Asp-75, Gly-86 to Asn-92, Asn-101 to Cys-106, Lys-119 to Leu-124, Pro-126 to Tyr-135, Ser-137 to Ser-150, His-161 to Ser-168, Asp-175 to Ser-182, Asn-189 to Lys-207, Pro-225 to Thr-234, His-240 to Gly-259, Glu-266 to Val-271, Asp-285 to Ala-290, Asn-321 to Ile-353.
876156	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5652 as residues: Lys-21 to Gly-26.
876170	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5656 as residues: Arg-15 to Arg-21.
876172	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5657 as residues: Trp-73 to Trp-80, Tyr-90 to Lys-97, Lys-100 to Trp-111.
876174	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5658 as residues: Gly-7 to Glu-12, Ser-16 to Gln-25.
876177	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5659 as residues: Phe-9 to Tyr-15.
876182	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5661 as residues: Pro-28 to Arg-34, His-66 to Pro-81, Ser-83 to Ala-93, Gly-98 to Lys-114.

876184	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5663 as residues: Asn-35 to Cys-40, Ser-75 to Phe-84.
876192	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5665 as residues: Thr-4 to Ser-14, Ile-83 to Ala-94.
876198	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5667 as residues: Pro-7 to Thr-17.
876200	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5668 as residues: Leu-43 to Pro-50.
876201	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5669 as residues: Pro-28 to Glu-37.
876206	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5670 as residues: Gly-29 to Asp-39.
876207	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5671 as residues: Arg-54 to Lys-95.
876208	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5672 as residues: Ser-44 to Leu-49, Lys-52 to Pro-57, Gly-65 to Phe-71, Asp-94 to Trp-99, Gly-137 to Asp-149, Ser-154 to Glu-159, Glu-168 to Ile-173.
876209	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5673 as residues: Gly-101 to Arg-107, Ser-112 to Cys-117.
876215	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5675 as residues: Phe-27 to Ile-34.
876224	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5677 as residues: Ser-58 to Gly-63, Thr-69 to Gly-76, Ser-107 to Thr-115, Ser-144 to Gly-151, Leu-175 to Trp-181.
876226	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5678 as residues: Arg-57 to Thr-62.
876228	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5679 as residues: Glu-7 to Ser-25, Lys-39 to Leu-46.
876229	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5680 as residues: Phe-48 to Ser-58.
876232	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5681 as residues: Thr-3 to Thr-8.
876238	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5683 as residues: Asn-30 to Lys-43, Pro-58 to Glu-65, Arg-77 to Asn-85.
876239	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5684 as residues: Thr-7 to Pro-15.
876259	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5685 as residues: Lys-1 to Gln-7, Gly-39 to Ile-50, Ile-68 to Cys-84, Leu-92 to Glu-99, Glu-109 to Glu-121, Pro-156 to Cys-172, Pro-174 to Thr-189, Arg-212 to Gln-227.
876260	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5686 as residues: Ala-40 to Ala-45.
876261	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5687 as residues: Arg-18 to Thr-31, Ala-39 to Gly-50, Ser-71 to Val-76.
876265	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5688 as residues: Thr-4 to Ser-9.

876266	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5689 as residues: Leu-26 to Lys-39.
876270	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5691 as residues: Pro-20 to Arg-27.
876274	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5692 as residues: Asn-52 to Ile-58.
876277	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5694 as residues: Arg-21 to Arg-30.
876280	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5696 as residues: His-16 to Phe-21.
876281	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5697 as residues: Gln-1 to Ser-8, Val-41 to Arg-47.
876282	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5698 as residues: Gln-1 to Val-6, Asp-8 to Thr-16.
876284	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5699 as residues: Ala-24 to Arg-30, Thr-88 to Pro-107.
876306	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5702 as residues: Gly-1 to Val-9, Pro-47 to His-57.
876308	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5703 as residues: Lys-28 to Ser-42, Gln-49 to Lys-57, Ser-76 to Gly-83, Glu-99 to Val-106, Gln-132 to His-142, Ala-202 to Trp-210, His-271 to Ile-287.
876309	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5704 as residues: His-58 to Ala-63, Arg-86 to Gly-92.
876322	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5705 as residues: Pro-33 to Arg-38, Thr-82 to Asp-88, Ala-103 to Lys-111, Lys-117 to Phe-122.
876326	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5706 as residues: Ser-15 to Asp-28, Glu-37 to Gly-42.
876330	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5708 as residues: Arg-41 to Lys-56.
876335	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5711 as residues: Glu-8 to Cys-16, Pro-22 to Gln-32, Lys-40 to Pro-49.
876340	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5712 as residues: Pro-1 to Glu-18, Gly-26 to Pro-33, Pro-66 to Gly-75.
876345	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5713 as residues: Arg-1 to Gly-10.
876354	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5714 as residues: Pro-12 to Thr-18.
876361	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5715 as residues: Arg-14 to Val-29.
876364	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5716 as residues: Gln-22 to Gly-28.
876370	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5717 as residues: Gly-4 to Arg-12, Gly-33 to Cys-46.
876372	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5718 as residues: Lys-30 to Glu-35.

876374	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5719 as residues: Ser-2 to Ser-8, Glu-26 to His-33, Ser-56 to Gly-61.
876380	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5722 as residues: Ser-11 to Pro-16.
876382	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5724 as residues: Glu-15 to Ser-20.
876383	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5725 as residues: Tyr-16 to Thr-21, Lys-33 to Gln-39.
876385	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5726 as residues: Leu-11 to Phe-16.
876395	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5729 as residues: Arg-7 to Ser-26.
876397	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5730 as residues: Pro-19 to Gln-25, Thr-41 to Pro-47.
876398	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5731 as residues: Glu-1 to Arg-7.
876400	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5733 as residues: Gln-13 to Trp-20, Gly-60 to Phe-65, Cys-69 to Trp-77.
876401	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5734 as residues: Gly-25 to Trp-30, Arg-37 to Gly-44, Ser-46 to Arg-59, Ser-70 to Ser-76, Leu-106 to Gly-112.
876404	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5736 as residues: Tyr-1 to Gly-17.
876405	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5737 as residues: Tyr-1 to Ala-6, Trp-30 to Ser-36, Asp-48 to Ile-62, Ile-91 to Ile-100, Asn-119 to Asn-128, Glu-146 to Glu-152.
876408	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5738 as residues: Gly-7 to Leu-15.
876409	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5739 as residues: Gly-10 to Asn-15.
876418	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5740 as residues: Pro-57 to Asp-63.
876420	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5742 as residues: Pro-6 to Ser-12.
876426	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5745 as residues: Phe-2 to Thr-12.
876428	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5747 as residues: Thr-4 to Trp-10, Pro-25 to Ala-31.
876431	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5748 as residues: Thr-1 to Gln-6, Lys-15 to Glu-23, Pro-39 to Ile-44, Asn-63 to Gln-71, Gln-101 to Arg-111, Leu-118 to Ser-124, Leu-141 to Val-146, Pro-154 to Pro-161, Ser-187 to Pro-192, Arg-207 to Met-245, Ala-253 to Ser-263.
876432	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5749 as residues: Lys-45 to Asn-55.
876435	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5750 as residues: Asp-84 to Asn-91.

876436	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5751 as residues: Pro-81 to His-89.
876440	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5752 as residues: Asp-1 to Leu-6, Glu-55 to Ser-60.
876441	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5753 as residues: Pro-14 to Leu-21, Cys-34 to Gly-39.
876448	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5756 as residues: Thr-1 to Glu-11, Thr-19 to Lys-30, Asn-32 to Glu-39, Leu-60 to Tyr-111, Ala-127 to Phe-132, Pro-184 to Thr-306.
876451	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5757 as residues: Thr-52 to Lys-59.
876452	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5758 as residues: Asn-1 to Arg-11, Val-23 to Ser-28, Asp-35 to Thr-40, Glu-116 to Arg-122, Leu-163 to Ser-170, Ile-267 to Ser-272.
876464	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5761 as residues: Thr-6 to Lys-11, Pro-58 to Ile-72, Ser-81 to Gly-94.
876465	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5762 as residues: Pro-2 to Trp-11, Pro-26 to Ala-32.
876469	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5763 as residues: Trp-1 to Leu-17.
876470	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5764 as residues: Pro-30 to Glu-41, Cys-62 to Trp-68, Leu-78 to Asn-97, Arg-131 to Asn-136.
876471	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5765 as residues: Val-7 to Leu-13, Glu-26 to Gln-32.
876472	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5766 as residues: Ser-91 to Gly-101.
876473	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5767 as residues: His-12 to His-22.
876476	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5770 as residues: Phe-2 to Trp-7, Cys-35 to Asn-46, Pro-55 to Asn-70, Pro-131 to Cys-137, Phe-141 to Thr-154, Ala-166 to Phe-177.
876481	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5772 as residues: Ala-87 to Ser-94, Asp-104 to Arg-112, Leu-114 to Asp-119, Ser-186 to Thr-202.
876483	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5773 as residues: Gly-1 to Pro-6.
876484	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5774 as residues: Met-2 to Leu-9, Lys-11 to Pro-28, Asp-57 to Leu-68, Gln-81 to Phe-118.
876487	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5775 as residues: Lys-1 to Ser-7.
876490	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5776 as residues: Glu-12 to Asp-17, Thr-26 to His-34, Asn-48 to Tyr-57.
876491	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5777 as residues: Arg-1 to Gln-11.
876494	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 5778 as residues: Asn-40 to Thr-45, His-210 to Pro-215, Glu-369 to Thr-375, Lys-383 to Leu-397, Pro-438 to Ile-447, Pro-510 to Tyr-520, Arg-528 to Arg-533, Thr-549 to Thr-555.
876495	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5779 as residues: Arg-11 to Arg-29, Arg-99 to Gly-105.
876496	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5780 as residues: Glu-1 to Gly-10.
876498	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5781 as residues: Ser-1 to Ser-14.
876499	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5782 as residues: Pro-19 to Tyr-25.
876504	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5784 as residues: His-7 to Asp-12, Glu-21 to Lys-26, Gln-79 to Ser-87.
876507	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5785 as residues: Pro-1 to Ser-12, Leu-26 to Gly-54, Thr-61 to Ala-73.
876513	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5787 as residues: Ser-3 to Gly-39, Trp-89 to Asp-96, Glu-103 to Asn-111, Leu-138 to Pro-145.
876518	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5788 as residues: Met-31 to Pro-38.
876524	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5789 as residues: Pro-26 to Gln-32.
876526	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5790 as residues: Met-7 to Tyr-13.
876530	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5791 as residues: Tyr-37 to Val-45.
876533	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5792 as residues: Lys-41 to Lys-47, His-52 to Gln-58, Gln-100 to Cys-106.
876535	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5794 as residues: Asp-1 to Asp-12.
876536	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5795 as residues: Gly-11 to Gly-28, Glu-35 to Ala-40, Leu-42 to Gly-51, Ser-65 to Cys-70.
876538	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5796 as residues: Tyr-5 to Thr-12.
876543	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5798 as residues: Gln-1 to Ala-9, Cys-56 to Gly-61, Trp-105 to Thr-110, Arg-150 to Thr-155, Leu-189 to Lys-195.
876544	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5799 as residues: Thr-15 to Asp-27.
876545	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5800 as residues: Arg-1 to Asp-7, Leu-19 to Lys-33, Ser-45 to Thr-54.
876546	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5801 as residues: Thr-15 to Lys-25, Pro-35 to Phe-42, Glu-58 to Thr-72, Glu-115 to Met-126, Gln-131 to Thr-139, Ser-142 to Glu-157,

	Pro-165 to Gln-188, Phe-284 to Lys-301.
876553	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5805 as residues: Arg-14 to Arg-19, Asn-27 to Val-32, Glu-68 to Thr-77, Gly-85 to Asp-90, Asp-221 to Gln-229, Thr-236 to Val-242, Thr-259 to Trp-266, Ser-268 to Asn-273, Asn-283 to Gly-288.
876558	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5807 as residues: Arg-22 to Gln-34.
876559	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5808 as residues: Asn-15 to Ser-20, Arg-100 to Phe-107, Glu-111 to Asp-118, Ile-122 to Val-127, Cys-219 to Val-227.
876560	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5809 as residues: Pro-7 to Ser-14, Thr-26 to Cys-51, Leu-55 to Tyr-64.
876572	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5810 as residues: Lys-16 to Lys-21.
876575	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5811 as residues: Pro-10 to Trp-19, Glu-47 to Gly-52, Tyr-75 to Gly-88, Met-119 to Asp-131.
876579	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5813 as residues: Ser-2 to Pro-21.
876581	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5815 as residues: Gly-32 to Gly-44, Pro-52 to Cys-60, Asp-63 to Leu-68, Lys-148 to Asn-160.
876583	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5816 as residues: Glu-19 to Cys-30.
876595	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5821 as residues: Asn-1 to Arg-8, Glu-64 to Thr-70.
876596	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5822 as residues: Lys-61 to His-66, Glu-70 to Tyr-78, Pro-90 to Ile-95, Val-118 to Asp-127, Asp-192 to Phe-199, Asn-274 to Met-279, Ser-281 to Arg-291, Thr-306 to Tyr-315, Lys-338 to Gln-343, Lys-350 to Asp-356, Pro-374 to Asp-380, Gly-398 to Pro-405, Lys-438 to Asn-446.
876597	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5823 as residues: His-1 to Ser-6, Glu-14 to Gly-22.
876600	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5824 as residues: Asp-22 to Pro-30, Ser-49 to Asn-57, Thr-76 to Ala-91.
876601	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5825 as residues: Leu-31 to Ser-41.
876602	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5826 as residues: Leu-11 to Arg-19, Arg-33 to Ala-38, Ala-40 to Gln-46, Pro-57 to Gly-62, Ser-70 to Arg-76, Thr-97 to Arg-103, Lys-119 to Lys-124.
876608	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5827 as residues: Val-10 to Gln-18.
876609	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5828 as residues: Leu-39 to Gln-52.
876610	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 5829 as residues: Ser-11 to Glu-20.
876612	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5830 as residues: Lys-1 to Asn-8, Glu-10 to Thr-15, Ser-22 to Gly-28, Pro-49 to His-54.
876622	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5832 as residues: Pro-46 to Leu-51.
876630	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5833 as residues: Gln-41 to Pro-46.
876633	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5835 as residues: Ala-1 to Leu-9, Ala-48 to Asp-55.
876638	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5837 as residues: Gln-1 to Arg-12, Asp-22 to Pro-44, Lys-52 to Asp-62, Pro-68 to Lys-93, Pro-99 to Pro-129, Ala-138 to Ser-150, Lys-156 to Val-194, Ile-197 to Glu-210, Ala-213 to Ala-287, Leu-289 to Lys-327, Lys-330 to Gly-340, Asp-344 to Gln-360, Ile-396 to Thr-401, Lys-409 to Asp-418, Met-450 to Ala-460, Glu-468 to Asp-481, Ala-490 to Ser-517, Asp-523 to Ser-555.
876643	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5838 as residues: Gln-1 to Ser-13.
876645	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5839 as residues: Gly-1 to Gln-20, Gly-22 to Glu-27, Arg-46 to Phe-52, Thr-64 to His-72, Pro-94 to Lys-109, Ser-143 to Ser-151.
876646	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5840 as residues: Ser-29 to Glu-34.
876647	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5841 as residues: Trp-41 to Ser-46, Glu-59 to Lys-66, Lys-75 to His-80.
876652	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5844 as residues: Phe-23 to Val-42.
876656	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5845 as residues: Ser-38 to Cys-51, Asn-93 to Asp-100.
876657	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5846 as residues: Pro-112 to Gly-118.
876660	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5847 as residues: Glu-20 to Arg-26, Leu-30 to Cys-36, Gln-49 to Ser-55, Lys-82 to Thr-90.
876666	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5848 as residues: Val-39 to Asn-46, Ser-95 to Asp-101, Lys-118 to Val-124.
876677	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5851 as residues: Asn-1 to Val-6, Phe-76 to Tyr-83, Gly-129 to Gln-135, Thr-145 to Asp-153, Ser-210 to Gln-220, Thr-230 to Asn-236, Lys-242 to Ala-248.
876680	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5852 as residues: Ser-1 to Thr-9, Ala-32 to Asn-37, Thr-40 to Tyr-49, Gln-71 to Thr-80.
876683	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5853 as residues: Pro-18 to Gly-29, Lys-67 to Lys-89.
876685	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 5854 as residues: Lys-19 to Asn-25, Leu-27 to Leu-38, Val-61 to Val-68, Leu-152 to Tyr-159, Glu-222 to Cys-228, Asp-260 to Leu-265.
876687	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5855 as residues: Ala-60 to Arg-65, Ala-82 to Arg-87.
876689	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5856 as residues: Arg-1 to Asn-9, Gln-20 to Asn-27, His-29 to Arg-34.
876690	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5857 as residues: Pro-15 to Asn-25, Glu-48 to Phe-59, Ser-69 to Arg-74, Ala-77 to Ser-82, Leu-99 to Asn-105, Ala-108 to Pro-124, Ser-137 to Phe-150, Ser-173 to Gly-178, Pro-186 to Pro-191, Ala-199 to Lys-213, Val-229 to Asp-238, Arg-272 to Asn-290.
876693	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5858 as residues: Glu-3 to Gly-12, Arg-20 to Gln-30, Leu-34 to Gln-39, Asp-51 to Arg-58, Gln-69 to Val-77, Gly-105 to Lys-117.
876696	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5859 as residues: Arg-1 to Arg-7, Gly-72 to Asp-78, Lys-83 to Gln-90.
876701	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5861 as residues: Thr-22 to Lys-31.
876716	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5862 as residues: Tyr-28 to Leu-33, Ala-70 to Lys-87, Glu-106 to Gly-124, Gly-127 to Glu-160, Leu-179 to Asp-194.
876719	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5863 as residues: Asn-19 to Ser-25, Gln-57 to Leu-66, Asp-76 to Ser-81, Glu-101 to Gln-106, Phe-121 to Asp-127, Ser-133 to Asp-146, Thr-186 to Lys-197, Arg-259 to Leu-266, Asn-268 to Leu-274.
876725	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5865 as residues: Thr-23 to Pro-34, Glu-39 to Asp-83, Asn-89 to Lys-99, Asp-118 to Asp-128, Asn-135 to Glu-150, Glu-153 to Gly-168, Gly-181 to Thr-187, Arg-200 to Asp-205, Arg-273 to Ile-279, Thr-295 to Asp-300, Thr-316 to Cys-321.
876726	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5866 as residues: Tyr-17 to Gly-22, Lys-29 to Tyr-34, Asp-39 to Asp-44, Leu-71 to Glu-76, Pro-164 to Gly-171.
876732	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5869 as residues: Ser-1 to Gln-6, Leu-57 to Phe-62, Arg-86 to Glu-91.
876744	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5871 as residues: Thr-98 to Ser-104, Thr-115 to Tyr-126, Gln-149 to Glu-164.
876745	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5872 as residues: His-1 to Gln-7, Trp-14 to Gln-29, Arg-41 to Pro-48, Leu-91 to His-97, Pro-99 to Ser-114, Ser-119 to Gly-124.
876747	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5873 as residues: Ala-13 to Arg-35, Pro-58 to Met-75, Asn-104 to Ser-119, Pro-144 to Ile-167, Lys-183 to Phe-224, Cys-246 to Gly-252, Lys-304 to Gly-320.
876750	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 5874 as residues: Ala-1 to Ser-6, Ser-29 to Ser-37, Gln-52 to Tyr-58.
876752	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5875 as residues: Pro-44 to Gly-51.
876753	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5876 as residues: Arg-5 to Arg-12.
876760	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5877 as residues: Thr-11 to Ala-16, Thr-85 to Glu-92, Asn-114 to Glu-122, Asp-150 to Gly-156.
876762	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5878 as residues: Pro-14 to Ile-24, Thr-35 to Pro-46.
876771	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5881 as residues: His-28 to Gly-33.
876773	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5882 as residues: Gly-3 to Thr-9, Glu-39 to Lys-48, Arg-134 to Lys-139, Pro-147 to Val-152, Thr-167 to Glu-172, His-190 to Gln-196.
876791	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5885 as residues: Pro-1 to Glu-20, Leu-79 to Ser-87, Lys-90 to Gly-96, Gln-109 to Thr-121, Val-133 to Gly-139.
876798	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5887 as residues: Thr-25 to Val-31, Lys-47 to Asp-62.
876802	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5888 as residues: Leu-2 to Thr-8, Asp-15 to Gly-26, Phe-64 to Ser-70, Pro-77 to Trp-82, Pro-85 to Lys-90.
876807	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5890 as residues: Lys-12 to Ser-18, Tyr-26 to Thr-33, Leu-71 to Thr-76, Pro-102 to Ser-110, Asp-114 to Gln-119, Glu-137 to Asp-159, Gly-162 to His-172, Thr-179 to Gly-194, Ala-198 to Asp-229.
876809	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5891 as residues: Arg-7 to Lys-13, Pro-28 to Cys-34, Gly-100 to Asn-109, Cys-155 to Arg-162, Glu-214 to Gln-219, Glu-240 to Asp-246, Gly-254 to His-265.
876817	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5894 as residues: Pro-22 to Asn-28, Pro-47 to Asn-57, Glu-92 to Gly-98, Pro-120 to Ile-135, Ala-138 to Cys-155, Pro-161 to Val-181, Ala-185 to Asp-196, Val-207 to Asn-213, Asn-219 to Asn-236, Asn-242 to Asn-250, Leu-252 to Asn-274, Ala-281 to Cys-295, Pro-297 to Cys-311, Pro-317 to Asn-339, Thr-417 to Tyr-423, Gln-443 to Gly-458, Thr-471 to His-476, Thr-484 to Gln-490, Asp-497 to Trp-511.
876823	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5896 as residues: Arg-1 to Trp-23, Pro-37 to Gly-47, Gly-50 to His-56, Phe-64 to Gly-74, Pro-76 to Ala-81, Pro-84 to Gly-95, Pro-101 to Pro-112, Lys-135 to Lys-146, Lys-159 to Asp-176.
876829	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5897 as residues: Pro-51 to His-56, Glu-69 to Asn-74, Gly-190 to Lys-199.
876830	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5898 as residues: Asp-27 to Gly-39.
876842	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5902 as residues: Glu-8 to Arg-13, Leu-17 to Val-23.

876856	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5903 as residues: Glu-63 to Asn-73, Pro-114 to Tyr-122, Ser-194 to Glu-201, Ile-263 to Ser-269.
876858	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5904 as residues: Asn-1 to Val-6, Lys-9 to Gln-16, Asn-47 to Glu-53, Asn-116 to Ser-121, Pro-130 to Thr-139, His-159 to Glu-165.
876865	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5905 as residues: Leu-26 to Asp-39, Asp-47 to Arg-54, Glu-62 to Val-72.
876866	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5906 as residues: Ser-1 to Gln-8, Val-40 to Ser-49, Arg-105 to Lys-110.
876870	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5907 as residues: Ser-25 to Trp-32.
876873	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5908 as residues: Gln-21 to Met-26, Gln-50 to Lys-61.
876876	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5909 as residues: Ala-8 to Arg-14, Ile-64 to Thr-69, Val-94 to Asp-101, His-112 to Gln-117, Tyr-139 to Glu-145, Tyr-195 to Cys-208, Gly-216 to Gly-223, Asp-297 to Ser-307, Gly-378 to Leu-383, Ile-391 to Pro-404, Asn-451 to Ser-466.
876878	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5910 as residues: Pro-32 to Arg-41.
876882	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5911 as residues: Thr-4 to Gly-13, Asp-20 to Val-25, Ala-46 to Asn-65, Gly-69 to Gly-75, Pro-82 to Gly-113, Pro-119 to Pro-174, Gly-181 to Gly-187, Tyr-190 to Tyr-212.
876886	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5912 as residues: Ser-9 to Arg-22, Gln-28 to Trp-34, Gly-36 to Leu-43, Arg-45 to Trp-52, Asp-56 to Leu-61, Ala-65 to Tyr-72, Leu-102 to Gly-109, Pro-111 to Ala-116, Ala-120 to Arg-125, His-129 to Gln-134, Pro-136 to Gly-145, Pro-167 to Thr-172, Glu-232 to Lys-239, Lys-253 to Asn-258, Leu-357 to Gly-362, Leu-371 to Gly-376.
876888	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5913 as residues: Glu-31 to Asp-39.
876890	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5914 as residues: Glu-91 to Pro-100, Tyr-122 to Thr-127, Thr-168 to Val-173, Thr-210 to Asp-215, Leu-219 to Gly-224, Gly-232 to Val-237.
876892	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5915 as residues: Ser-8 to Ser-20.
876901	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5916 as residues: Tyr-130 to Glu-136, Arg-148 to His-159, Pro-214 to Leu-221, His-224 to Gly-229, Glu-238 to Glu-246, Gln-331 to Trp-343.
876904	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5918 as residues: Val-61 to Gln-69, Gln-106 to Thr-111.
876905	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5919 as residues: Arg-1 to Arg-7, Pro-29 to Lys-56, Asp-103 to Arg-108, Tyr-122 to Ser-127, Gly-219 to Glu-227, Asp-250 to Glu-255,

	Glu-294 to Pro-301, Ala-321 to Tyr-327, Arg-367 to Pro-373, Glu-396 to Asn-405, Gly-411 to Arg-418, Asn-433 to Lys-441.
876909	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5920 as residues: Ala-32 to Ala-40, Glu-93 to Phe-103, Lys-173 to Thr-189.
876912	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5921 as residues: Glu-40 to Pro-47, Lys-56 to Trp-62.
876920	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5923 as residues: Arg-1 to Gly-15, Ser-42 to Trp-51, Pro-59 to Arg-64.
876921	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5924 as residues: Tyr-1 to Leu-6.
876923	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5925 as residues: Pro-6 to Cys-14, Glu-33 to Leu-38, Val-209 to Lys-216, Pro-270 to Gln-278, His-321 to Thr-330.
876936	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5928 as residues: Ala-54 to His-67, Pro-69 to Lys-86.
876940	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5930 as residues: Ala-1 to Asp-29, Pro-51 to His-59, Asn-67 to Asp-73.
876941	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5931 as residues: Pro-16 to Arg-28.
876942	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5932 as residues: Glu-1 to Gln-6, Val-8 to Trp-15.
876943	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5933 as residues: Gly-1 to Gln-9, Asn-11 to Arg-16, Cys-28 to His-33, Pro-51 to Pro-57, Glu-66 to Glu-72, Pro-84 to Asp-89, Pro-104 to Asp-109, Glu-122 to Thr-132.
876944	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5934 as residues: Arg-3 to Gly-11.
876945	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5935 as residues: Pro-15 to Pro-24.
876946	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5936 as residues: Ser-8 to Ser-14.
876947	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5937 as residues: Gly-27 to Ala-34.
876949	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5938 as residues: Pro-5 to His-14, Arg-38 to Gln-43, Leu-80 to Arg-86.
876952	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5939 as residues: Ser-8 to Thr-18, Pro-52 to Ala-61, Pro-67 to Gly-72, Ala-81 to Thr-88, Glu-105 to Thr-120.
876953	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5940 as residues: Gly-1 to Asp-12, Ser-64 to Trp-74, Met-82 to Tyr-88, Phe-101 to Cys-106, Tyr-120 to Lys-132.
876954	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5941 as residues: Pro-1 to Ile-12, Asp-30 to Tyr-35, Leu-38 to Pro-45, Lys-54 to Thr-60, Thr-75 to Leu-80, Asp-92 to Tyr-100, Ile-133 to Thr-138, Thr-194 to Glu-199, Asp-233 to Leu-239, Met-243 to Ala-

	251, Asp-254 to Glu-261.
876957	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5942 as residues: Lys-71 to Asn-88, Ala-115 to Cys-130, Ala-175 to Arg-182.
876958	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5943 as residues: Gln-1 to Pro-8.
876963	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5946 as residues: Val-16 to Ser-21, Ala-60 to Lys-72.
876964	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5947 as residues: Thr-6 to Lys-13, Met-16 to Glu-36, Lys-59 to Phe-65, Leu-71 to Gln-77.
876966	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5949 as residues: Lys-13 to Trp-19, Ser-25 to Gln-32, Glu-53 to Gln-58, Arg-108 to Gly-113.
876967	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5950 as residues: Lys-1 to Asp-9, Arg-16 to Gly-21, Cys-51 to Val-59, Asp-65 to Ser-71, Thr-79 to Asn-90, Asn-99 to Asn-111, Ser-149 to Pro-156.
876968	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5951 as residues: Asn-44 to Tyr-49, Gly-71 to Glu-79.
876969	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5952 as residues: Arg-74 to Arg-79.
876975	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5954 as residues: Phe-12 to Ile-19, Arg-25 to Arg-31.
876976	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5955 as residues: Asn-78 to Gln-92.
876977	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5956 as residues: Asn-1 to Glu-8, Ala-38 to Gly-46, Gln-58 to Asp-71, Ala-75 to Cys-103, Met-106 to Ala-140, Gln-153 to Ile-159.
876981	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5959 as residues: Gln-40 to Lys-45.
876983	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5960 as residues: Leu-37 to Pro-42.
876984	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5961 as residues: His-5 to Thr-11, Arg-71 to Pro-77.
876985	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5962 as residues: Tyr-7 to Gly-28, Arg-38 to Asp-65, Asp-78 to Ser-90, Ser-92 to Ser-115, Asp-117 to Ser-132, Val-148 to Leu-153, Lys-155 to His-168.
876987	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5963 as residues: Lys-30 to Thr-35, Ser-49 to Tyr-55.
876989	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5964 as residues: Gly-4 to Gly-10, Glu-17 to Gly-28, Met-35 to Asp-41, Glu-79 to Gln-85, Gln-102 to Gly-110.
876992	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5967 as residues: Ser-15 to Pro-21.
876993	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5968 as residues: His-44 to Gln-52, Pro-55 to Lys-72, Ser-87 to Ser-93, Arg-105 to Leu-111, Phe-119 to Lys-124.

876994	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5969 as residues: Leu-28 to Glu-33, Met-54 to Cys-60.
876998	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5971 as residues: Glu-1 to Pro-25, Gly-30 to Ala-54, Asn-65 to Asn-82, Leu-89 to Ser-97.
877000	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5972 as residues: Ala-1 to Asn-6, Val-8 to Tyr-20.
877002	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5974 as residues: Ser-32 to Gly-53, Thr-61 to Ser-70.
877005	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5976 as residues: Gly-12 to Gly-22.
877006	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5977 as residues: Glu-8 to Ser-14, Thr-26 to Asn-40.
877007	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5978 as residues: Glu-31 to Leu-38.
877008	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5979 as residues: Ser-37 to Ser-47, Gln-58 to Thr-69, Val-72 to Gln-77, Gly-125 to Lys-155.
877010	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5981 as residues: Gly-20 to Ser-29.
877011	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5982 as residues: Ser-30 to Trp-36.
877014	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5985 as residues: Asp-1 to Arg-31, Lys-35 to Lys-44, Glu-55 to Leu-61, Thr-71 to Asp-76, Ile-82 to Asn-101.
877015	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5986 as residues: Lys-1 to His-12, Ser-26 to Thr-31, His-54 to Val-60.
877018	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5987 as residues: Gly-9 to Glu-16, Asn-46 to Glu-54.
877019	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5988 as residues: Lys-24 to Glu-38, Arg-48 to Ala-54, Gly-61 to Ala-67.
877022	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5990 as residues: Arg-10 to Gly-15, Thr-55 to Lys-64.
877024	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5992 as residues: Thr-19 to Pro-26.
877025	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5993 as residues: Gly-19 to Asn-27.
877026	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5994 as residues: Met-27 to Asn-34, Val-57 to Glu-84, Glu-86 to Ala-100, Asp-122 to Ala-128.
877027	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5995 as residues: Gln-36 to Ser-42.
877030	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 5997 as residues: Glu-30 to Ala-35, Leu-39 to Ser-44, Pro-50 to Asp-57.
877037	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6001 as residues: Gln-61 to Lys-67.

877044	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6003 as residues: Arg-22 to Gly-27, Ser-34 to Gly-39.
877046	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6004 as residues: Phe-65 to Trp-73, Arg-87 to Gly-92, Gly-107 to Lys-112, Pro-177 to Thr-186, Glu-251 to Arg-256, Phe-282 to Lys-287.
877047	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6005 as residues: Tyr-2 to Gly-8.
877049	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6006 as residues: Pro-2 to Pro-11.
877050	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6007 as residues: Ser-36 to Lys-42.
877051	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6008 as residues: Gln-5 to Arg-12, Tyr-32 to Ser-43.
877056	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6010 as residues: Pro-52 to Val-57, Asp-59 to Gln-69.
877058	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6012 as residues: Thr-13 to Pro-20.
877059	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6013 as residues: Leu-30 to Ser-38.
877063	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6014 as residues: Asp-4 to Ala-15.
877066	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6016 as residues: Gln-1 to Trp-11, Pro-47 to Tyr-53.
877067	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6017 as residues: Pro-11 to Asp-16, Arg-23 to Gln-29.
877068	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6018 as residues: Lys-26 to Arg-32.
877070	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6020 as residues: Arg-33 to Leu-40.
877071	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6021 as residues: Pro-23 to Asn-31, Leu-33 to Phe-38.
877073	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6022 as residues: Ser-1 to Ser-17.
877087	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6027 as residues: Arg-1 to Met-6, Thr-34 to Glu-54, Glu-58 to Asn-63.
877088	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6028 as residues: Thr-6 to Gly-13, Trp-20 to Thr-36.
877092	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6029 as residues: Arg-17 to Gly-23.
877093	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6030 as residues: Pro-33 to Cys-43.
877094	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6031 as residues: Pro-9 to Tyr-17, Gln-29 to Tyr-38, Ala-47 to Glu-55.
877096	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6033 as residues: Lys-9 to Ser-17.
877097	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 6034 as residues: Phe-34 to Ser-39, Glu-63 to Phe-74, Leu-78 to Pro-83.
877098	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6035 as residues: Lys-1 to Asp-8.
877099	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6036 as residues: Pro-10 to Gly-17, Tyr-23 to Ser-28.
877101	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6037 as residues: Asp-22 to Cys-28, Gly-87 to Leu-93, Lys-128 to Asn-151.
877105	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6039 as residues: Pro-48 to Cys-53.
877106	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6040 as residues: Gln-3 to Ile-12.
877110	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6041 as residues: Val-6 to Ala-13.
877111	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6042 as residues: Phe-56 to Asn-72, Gln-84 to Leu-93, Ser-96 to Pro-109, Pro-116 to Glu-126.
877114	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6044 as residues: Lys-13 to Lys-21.
877119	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6045 as residues: Ala-16 to Ser-22.
877120	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6046 as residues: Pro-1 to Gly-14, Gly-33 to Ser-40, Gln-80 to Ser-101.
877121	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6047 as residues: Arg-34 to Ser-40.
877123	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6049 as residues: Thr-33 to Asp-38.
877126	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6050 as residues: Gly-10 to Leu-22, Gly-47 to Lys-62.
877132	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6054 as residues: Ser-2 to Lys-8.
877133	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6055 as residues: Thr-1 to Asp-8.
877135	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6057 as residues: Leu-7 to Leu-13, Pro-15 to Cys-28, Ser-50 to Lys-56.
877137	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6058 as residues: Glu-65 to Arg-72.
877138	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6059 as residues: Lys-15 to Thr-21.
877140	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6061 as residues: Ile-45 to Phe-51.
877142	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6062 as residues: Thr-5 to Ser-12.
877143	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6063 as residues: Arg-1 to Leu-6.
877148	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 6067 as residues: Leu-32 to Trp-37.
877149	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6068 as residues: Lys-72 to Gln-86.
877153	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6069 as residues: Cys-40 to Cys-46.
877154	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6070 as residues: Asn-24 to Phe-29, Thr-45 to Lys-50.
877165	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6074 as residues: Arg-6 to Lys-11, His-20 to Asn-25.
877166	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6075 as residues: Tyr-1 to Arg-7.
877167	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6076 as residues: Glu-25 to Asn-34.
877168	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6077 as residues: Tyr-1 to Ile-6, Val-17 to Ser-23, Thr-35 to His-40.
877169	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6078 as residues: Pro-1 to Met-12.
877170	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6079 as residues: Ser-4 to Lys-9.
877171	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6080 as residues: Val-10 to Leu-15, Arg-34 to Leu-40.
877173	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6081 as residues: Pro-18 to Gly-31.
877174	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6082 as residues: Lys-16 to Gln-21.
877175	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6083 as residues: Glu-2 to Ser-9.
877181	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6085 as residues: Glu-16 to Glu-23.
877187	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6088 as residues: Asp-41 to Ile-50, Thr-73 to Val-89, Gln-118 to Asp-123.
877194	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6091 as residues: Gly-53 to Asp-63.
877195	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6092 as residues: Pro-17 to Ile-24, Pro-28 to Phe-34.
877200	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6093 as residues: Thr-29 to Lys-35, Asp-44 to Cys-49.
877202	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6094 as residues: Gly-17 to Ala-23, Leu-52 to Asn-58.
877205	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6095 as residues: Lys-12 to Asp-18, Leu-40 to Arg-67, Val-75 to Asp-84.
877207	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6097 as residues: Ala-19 to Arg-29.
877208	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6098 as residues: Tyr-4 to Gln-9.
877211	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 6099 as residues: Asp-12 to Arg-17, Asp-34 to Gln-43, Asn-78 to Glu-84, Ser-99 to Ala-105, Ser-108 to His-113, Ile-115 to Gly-122, Phe-132 to Arg-148.
877212	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6100 as residues: Gln-1 to Ser-9.
877213	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6101 as residues: Arg-42 to Gln-53, His-56 to Ala-62, Asn-73 to Pro-81.
877214	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6102 as residues: Ser-15 to Cys-23.
877218	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6104 as residues: Lys-33 to Phe-40, Pro-64 to Arg-72, Arg-105 to Gly-110.
877220	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6105 as residues: Gly-1 to Thr-14, Ala-27 to Leu-32, Pro-47 to Pro-54.
877230	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6108 as residues: Thr-1 to Asn-8.
877231	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6109 as residues: Gly-1 to Ser-20, Phe-29 to Asn-37, Asn-55 to Tyr-64, Ala-69 to Asp-78, Tyr-82 to Ala-91, Lys-100 to Glu-122.
877232	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6110 as residues: Lys-41 to Ile-47.
877233	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6111 as residues: Ile-11 to Phe-16, Tyr-27 to Pro-33.
877234	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6112 as residues: Ala-13 to His-18, Gly-24 to Thr-29, Pro-31 to Gly-39, Pro-49 to Asp-56, Trp-64 to Asp-72, Pro-74 to Asp-80.
877235	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6113 as residues: Thr-6 to Gly-12, Pro-41 to Asp-48, Gly-54 to Phe-62, His-94 to Tyr-102, Ser-108 to Gly-123, Gln-130 to Asn-136, Tyr-169 to His-175, Phe-188 to Arg-195, Trp-232 to Ile-237.
877240	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6115 as residues: His-1 to Leu-8, Ala-42 to Arg-50, His-74 to Tyr-81.
877242	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6116 as residues: Asp-25 to Asn-30.
877250	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6118 as residues: Thr-11 to Cys-22, Gly-29 to Gly-37, Arg-74 to Asn-91, Phe-110 to Pro-119, Thr-144 to Gln-149, Tyr-165 to Gly-171, Pro-190 to Ser-196.
877251	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6119 as residues: Ala-5 to Ser-11, Thr-32 to Thr-37, Gln-46 to Asp-57, Ala-70 to Gly-78.
877254	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6120 as residues: Val-50 to Tyr-55, Thr-63 to Thr-68, Phe-77 to Gly-92, Arg-112 to Lys-119.
877258	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6124 as residues: Thr-1 to Ser-6, Thr-40 to Trp-49, Asn-65 to Lys-72.

877263	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6126 as residues: Asp-1 to Ser-16, Pro-21 to Glu-26, Pro-46 to Asn-55, Thr-74 to Leu-86, Ser-96 to Asp-105.
877264	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6127 as residues: Thr-1 to Arg-6, Ser-14 to Arg-20.
877272	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6128 as residues: Ile-55 to Leu-69, Thr-84 to Pro-94, Pro-104 to His-120.
877274	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6129 as residues: Glu-50 to Pro-58, Ile-88 to Gly-97, Pro-107 to Gly-116, Gln-136 to Gly-142, Asp-164 to Glu-176.
877275	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6130 as residues: Pro-1 to Gln-19, Cys-27 to Thr-34, Ile-49 to Trp-56.
877281	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6132 as residues: Lys-17 to Thr-23.
877282	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6133 as residues: Ala-1 to Lys-7, Asp-12 to Phe-17, Ile-24 to Glu-43.
877283	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6134 as residues: Lys-18 to Ile-23.
877284	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6135 as residues: Ile-41 to Trp-46, Glu-64 to Gly-80, Glu-134 to Gly-141, Phe-143 to Ser-158, Gln-207 to Asp-212.
877285	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6136 as residues: His-1 to Leu-11.
877290	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6140 as residues: Pro-34 to Tyr-40, Ser-67 to Trp-73, Asp-103 to Phe-109, Gln-130 to Gly-135, Trp-188 to Trp-197, Leu-327 to Asn-333, Gly-401 to Asn-407, Asn-473 to Val-483, Ser-523 to Gln-529, Arg-538 to Ser-544, Ala-563 to Ser-573, Gln-581 to Thr-592.
877295	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6141 as residues: Gln-54 to Leu-66, Pro-74 to Asp-79, Val-104 to Leu-112, Asn-114 to Asn-122, Glu-141 to Lys-152, Pro-265 to Leu-271, Phe-275 to Ser-280, Glu-298 to Ala-304, Arg-317 to Leu-323, Gln-332 to Tyr-337, Gln-342 to Arg-352.
877298	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6142 as residues: Ser-60 to Gly-66.
877301	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6144 as residues: Gln-17 to Lys-24, Ala-28 to Cys-35.
877310	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6145 as residues: Met-2 to Leu-12, Ser-16 to Asp-23, Gly-38 to Lys-45.
877319	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6146 as residues: Ala-30 to Glu-44.
877321	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6148 as residues: Gln-1 to Arg-7.
877326	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6150 as residues: Thr-25 to Asp-31.

877327	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6151 as residues: Thr-3 to Ser-10.
877332	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6154 as residues: Gly-26 to Arg-43.
877333	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6155 as residues: Pro-10 to Trp-19.
877334	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6156 as residues: Ala-18 to Ala-32, Thr-52 to Ser-60.
877336	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6157 as residues: Cys-10 to Phe-17.
877340	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6160 as residues: Ser-32 to Arg-38, Ala-72 to Lys-79, Arg-103 to Phe-111.
877344	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6161 as residues: His-41 to Thr-48.
877346	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6162 as residues: Ala-66 to Gln-78.
877355	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6165 as residues: Ser-12 to His-21, Pro-59 to Asp-69.
877356	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6166 as residues: Ser-12 to His-21, Pro-59 to Glu-68.
877361	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6168 as residues: Pro-1 to Ser-7, Thr-45 to Leu-63, Arg-113 to Thr-118, Pro-172 to Gly-182.
877370	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6170 as residues: Asp-17 to Gly-23, Lys-89 to Asp-94, Lys-129 to Asp-134, Leu-195 to Glu-204, Asn-325 to Val-336.
877373	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6171 as residues: His-8 to Gly-18, Gln-56 to Arg-61, Arg-160 to Pro-170, Ala-200 to Ser-212, His-225 to Lys-231, Gly-245 to Lys-254, Tyr-257 to Tyr-277, Pro-279 to Thr-287, Pro-305 to Gly-327, Tyr-342 to Glu-348.
877375	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6172 as residues: Gln-1 to Ser-22, Lys-40 to Phe-48, Leu-52 to His-57.
877377	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6173 as residues: Ser-27 to Thr-42, Lys-71 to Lys-85, Gly-99 to Arg-105.
877378	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6174 as residues: Lys-25 to Lys-39, Gly-53 to Arg-59, Ser-172 to Val-181.
877380	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6175 as residues: Glu-7 to Arg-20, Thr-28 to Trp-44, Ser-110 to Lys-118, Pro-124 to Arg-130, Ala-137 to Asn-147.
877384	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6176 as residues: Thr-2 to Leu-9, Thr-12 to Gly-17, Glu-26 to Ser-61, Asn-70 to Cys-80, Cys-84 to Ala-91, Lys-111 to Ser-119, Asn-170 to Gln-183, Ser-203 to Lys-210, Gln-216 to Pro-229, Arg-238 to Trp-255, Ile-257 to Phe-269.

877387	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6177 as residues: Asp-12 to Tyr-18, Pro-57 to Leu-63, Glu-90 to Ala-96, Gly-102 to Val-111, Gln-123 to Ile-129, Asp-143 to Ala-150, Lys-156 to Arg-161, Thr-213 to Cys-220, Arg-256 to Tyr-261, Ser-265 to Asp-274, Asp-290 to Ser-297, Val-307 to Arg-313, Asp-324 to Lys-337, Ser-438 to Arg-443, Asn-580 to Glu-585.
877388	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6178 as residues: Gly-15 to Asn-22.
877390	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6179 as residues: Cys-7 to Gly-24, Thr-31 to Val-53, Trp-102 to Glu-108, Thr-118 to Gly-124.
877393	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6180 as residues: Glu-4 to Trp-9.
877406	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6182 as residues: Asn-1 to Glu-27, Lys-37 to Lys-46, Arg-59 to Lys-83, Asn-89 to Phe-95, His-102 to Asn-107, Ser-155 to Ile-168, Pro-175 to Gln-188, Asn-201 to Pro-211, Ala-234 to Ile-239, Asn-249 to Val-257, Pro-261 to Gly-275.
877408	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6183 as residues: Gln-1 to Pro-16, Pro-21 to Pro-30, Gly-47 to Gly-65, Tyr-78 to Leu-86, Glu-88 to Pro-104, Glu-118 to Ala-131, Ala-143 to Trp-150, Asp-152 to Ser-157, Ser-180 to Trp-187, Ser-190 to Pro-197, Ala-211 to Asn-219, Asp-252 to Leu-257, Thr-287 to Val-295.
877411	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6184 as residues: His-20 to Gln-25, Asn-36 to Ser-56.
877630	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6186 as residues: Gln-40 to Phe-45.
878274	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6191 as residues: Pro-6 to Trp-14, Tyr-19 to Leu-26, Pro-56 to His-66, Tyr-70 to Arg-80, Thr-83 to Leu-100, Cys-107 to Phe-112, Lys-137 to Arg-148, Pro-155 to Leu-162.
878374	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6192 as residues: Arg-20 to Leu-28, Phe-57 to Arg-79.
878403	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6193 as residues: Ser-2 to Thr-8.
878433	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6194 as residues: Asn-17 to His-24, Pro-97 to Glu-111.
878436	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6195 as residues: Ser-18 to Thr-25.
878560	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6196 as residues: Thr-33 to Pro-40, Asp-62 to Glu-67, Ser-104 to Phe-109.
878800	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6197 as residues: Leu-24 to Arg-30.
878909	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6198 as residues: Pro-14 to Ser-19, His-40 to Trp-49.
878917	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6199 as residues: Glu-26 to Thr-32, Ser-41 to Pro-46, Leu-107 to Glu-115.
879009	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 6201 as residues: Trp-60 to His-68, Pro-99 to Gly-106.
879234	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6202 as residues: Ser-46 to Thr-64, Thr-69 to Gly-79, Ser-102 to Arg-115, Leu-137 to Thr-144, Ala-146 to Pro-153, Pro-163 to Arg-180, Cys-209 to His-229.
879386	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6203 as residues: Pro-3 to Cys-11, Pro-70 to Phe-83, Ser-101 to Leu-107, Glu-110 to Pro-116, Lys-153 to Arg-158.
879484	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6204 as residues: Lys-44 to His-50, Thr-110 to Pro-116, Lys-178 to Gln-183, Pro-196 to Lys-205, Arg-214 to Thr-220, Asp-295 to Leu-301, Pro-316 to Glu-324, Glu-331 to Tyr-336, Gly-347 to Val-354.
879595	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6205 as residues: Pro-7 to Ser-15, Gly-49 to Ala-55, Gln-74 to Pro-86.
879661	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6206 as residues: Arg-10 to Arg-20, Gly-48 to Val-53, Glu-69 to Asp-76, Glu-116 to Glu-122, Glu-132 to Trp-143, Asp-166 to Asn-175, Arg-191 to Asn-197, Gln-205 to Gly-233, Lys-235 to Ala-274.
880071	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6208 as residues: Ser-36 to Ser-41, Ser-77 to Gln-83.
880074	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6209 as residues: Ser-7 to Gln-12, Gly-25 to Gly-31, Gly-71 to Gly-84, Leu-147 to Glu-164, Trp-172 to Leu-180.
880418	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6210 as residues: Ser-56 to Val-64, Lys-66 to Cys-73.
880649	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6212 as residues: His-28 to Gly-35, Gln-141 to His-147, Glu-232 to Gln-237, Ala-264 to Glu-269.
880694	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6213 as residues: Glu-21 to Glu-27, Arg-34 to Ile-41, Leu-83 to Ala-93, Pro-120 to Glu-130.
880747	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6214 as residues: Pro-16 to Phe-23, Gln-45 to Cys-50, Asn-66 to Asn-73, Ile-98 to His-105, Pro-183 to Pro-190, His-206 to Ser-212, Thr-295 to Pro-316, Ser-364 to Trp-370, Gln-385 to Asn-396.
880994	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6216 as residues: Ile-32 to Tyr-47.
881105	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6220 as residues: Arg-9 to Gln-35, Ile-113 to Gly-120.
881219	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6221 as residues: Ser-17 to Thr-25, Lys-39 to Thr-48, His-53 to Arg-60, Pro-67 to Asn-72, Thr-157 to Phe-165, Gln-212 to Glu-221, Gly-241 to Ser-260, Thr-294 to Phe-300, Ile-319 to Lys-328, Ser-338 to Lys-343, Leu-383 to Phe-388, Gly-430 to Asp-441, Ser-466 to Glu-475, Gln-541 to Pro-554, Val-583 to Thr-595, Leu-598 to Arg-603, Gln-608 to Gln-614, Asp-639 to Asn-648, Asp-654 to Phe-667, Lys-676 to Val-704, Lys-725 to Ser-731, Pro-739 to Ala-763, Asp-772 to Gly-778.
881221	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6222 as residues: Ile-1 to Lys-11, Asn-59 to Phe-65, Phe-70 to

	Asn-79, Lys-156 to Glu-162, Pro-168 to Asp-175, Pro-213 to Leu-219, Asn-246 to Leu-266, Ser-275 to Asp-286, Gln-334 to Leu-345.
882330	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6223 as residues: Arg-20 to Ser-27, Glu-40 to Glu-50.
882715	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6224 as residues: Glu-4 to Asn-14, Gln-66 to Gly-73, Leu-88 to Leu-97, Val-101 to Gln-107.
882729	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6225 as residues: Arg-7 to Gly-12, Met-42 to Ser-58, Gln-65 to Asn-73, Glu-91 to Ala-99, Pro-103 to Tyr-109, Arg-174 to Ala-179, His-189 to Gln-196, Asn-208 to Pro-219.
882762	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6226 as residues: Arg-8 to Asn-30, Ser-37 to Gln-42, His-74 to Leu-82, Arg-92 to His-97, Gln-114 to Leu-119, Gly-131 to Gly-137.
883172	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6227 as residues: His-1 to Arg-10.
883371	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6230 as residues: Asp-24 to Trp-41, Tyr-106 to Lys-114, Ala-161 to Glu-167, Pro-182 to Leu-190, Ala-193 to Pro-200, Leu-205 to Tyr-212, Pro-240 to Lys-252, Pro-254 to Lys-262, Leu-293 to Leu-303.
883753	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6231 as residues: Gly-156 to Met-161, Cys-186 to Lys-197.
883799	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6232 as residues: Ser-1 to Glu-18, Val-79 to Glu-88.
883945	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6233 as residues: Ser-21 to Arg-28.
883971	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6234 as residues: Ser-19 to Gly-24, Gly-54 to Ser-59.
884038	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6235 as residues: Pro-18 to Asn-25, Ala-44 to Asn-50, Arg-56 to Lys-64, Gly-76 to Gly-85, Lys-92 to Leu-98, Gly-116 to Gly-121, Gln-132 to His-138, Thr-159 to Asp-167.
884095	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6236 as residues: Arg-50 to Thr-56, Pro-116 to Arg-121, Lys-129 to Phe-136, Glu-139 to Leu-144, Lys-156 to Leu-162.
884161	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6237 as residues: Asn-16 to Tyr-23, Glu-47 to Trp-56, Ser-90 to Lys-96, Ala-126 to Glu-136, Pro-138 to Lys-149, Glu-181 to Gly-186, Trp-208 to Lys-219, Arg-347 to Ala-358, Leu-370 to Lys-381, Thr-408 to Ile-415, Pro-425 to Leu-437, Gln-450 to Asn-455.
884168	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6238 as residues: Glu-94 to Tyr-102, Pro-105 to Asn-112, Thr-121 to Gly-137, Glu-157 to Gly-162, Glu-179 to Phe-186, Cys-211 to Thr-222, Ser-240 to Lys-245, Thr-262 to Asn-279, Arg-288 to Pro-306, Asn-332 to Gln-339, Ser-375 to Leu-382, Arg-408 to Gly-415, Asp-423 to Thr-428, Ser-471 to Asn-476, Pro-545 to Gly-551, Ser-606 to Pro-616, Ala-662 to Gly-667, Thr-675 to Tyr-682, Glu-714 to Trp-720, Pro-722 to Val-732, Pro-787 to Thr-795, Arg-811 to Glu-816, Gln-880 to Thr-891.
884215	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 6239 as residues: Met-10 to Gln-18, Pro-23 to Leu-31, Glu-46 to Arg-51, Phe-135 to Pro-143, His-218 to Asp-227, Pro-244 to Met-250, Lys-258 to Asp-263, Pro-266 to Leu-276, Pro-286 to Asp-293.
884529	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6241 as residues: Arg-8 to Ser-15, Gln-89 to Gln-95, Leu-109 to Tyr-115, Glu-126 to Arg-133.
884719	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6242 as residues: Arg-4 to Ala-10, Arg-40 to Gly-45, Asp-86 to Tyr-91, Pro-100 to Phe-113.
885350	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6243 as residues: Arg-15 to Pro-21, Cys-29 to Cys-41, Pro-52 to Leu-63, Pro-98 to Ser-108, Tyr-113 to Cys-118, Cys-124 to Asp-129, Cys-180 to Gln-187, Cys-247 to Cys-259, Ser-279 to Trp-286, Cys-296 to Cys-302, Pro-304 to Cys-309, Ser-343 to His-348, Gln-367 to Lys-373.
885476	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6244 as residues: Lys-28 to Glu-51, Lys-123 to Leu-133.
885484	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6245 as residues: Arg-1 to Glu-10, Gly-22 to Gly-27.
886505	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6248 as residues: Ser-64 to Gln-70, Ala-75 to Leu-80, His-82 to Gly-87, Ser-121 to Lys-137.
886788	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6250 as residues: Lys-60 to Lys-65, Lys-78 to Lys-94, Leu-116 to Gln-123.
887098	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6252 as residues: Pro-1 to Ala-9, Val-56 to Val-63, Gly-86 to Glu-91.
887114	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6253 as residues: Glu-38 to Arg-52, Ser-56 to Val-62.
887155	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6254 as residues: Thr-3 to Pro-9, Pro-18 to Gly-25, Ala-30 to Gly-36, Arg-41 to Asp-56, Ala-60 to Pro-68, Met-99 to Leu-128, Thr-143 to Phe-157.
887172	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6255 as residues: Cys-5 to Ser-14, Val-83 to Ser-88.
887192	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6256 as residues: Glu-29 to Cys-39, Val-46 to Ser-52, Asn-58 to Gly-65, Cys-68 to His-82, Tyr-84 to Gly-94, Leu-122 to Trp-138, Ala-158 to Leu-170, Gly-175 to Arg-182, Tyr-203 to Ser-210, Gly-246 to Met-258, Arg-288 to Gln-296.
887280	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6257 as residues: Asn-1 to Gly-15, Pro-18 to Asn-28, Gln-35 to Glu-40, Arg-60 to Arg-69.
887399	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6258 as residues: Pro-8 to Gly-18, Ala-94 to Gly-99, Asn-107 to Arg-112, Phe-161 to Arg-166, Thr-196 to Phe-201, Tyr-309 to Gly-316, Leu-326 to Arg-331.
887535	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6261 as residues: Glu-26 to Gly-32, His-73 to Arg-79.

887803	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6262 as residues: Ala-1 to Gln-7, Lys-24 to Ser-30, Pro-44 to Ser-49, Ser-99 to Ser-105.
887857	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6263 as residues: Pro-1 to Ser-6, Pro-25 to Cys-31, Arg-142 to Lys-150, Pro-223 to Gly-230, Ala-233 to Val-247.
887892	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6264 as residues: Ser-10 to Ile-15, Val-60 to Arg-66, Tyr-114 to Leu-128.
887936	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6265 as residues: Leu-1 to Cys-6, Lys-46 to Thr-53, Ala-56 to Glu-63.
887996	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6266 as residues: Ala-1 to Gly-6, Pro-9 to Pro-24, Gln-70 to Tyr-82, Glu-127 to Ser-134.
888051	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6268 as residues: Trp-45 to Trp-56, Thr-58 to Asp-73, Thr-126 to Arg-133, Phe-148 to Ser-155, Val-208 to Gly-223.
888153	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6270 as residues: Gly-5 to Leu-12, Tyr-18 to Asp-25, Ile-88 to Ala-125, Ser-129 to Tyr-141, Gln-191 to Gln-196, Thr-290 to Asn-296, Thr-301 to Thr-309, Leu-360 to Ala-365, Leu-367 to Gly-378, Pro-398 to Gly-418, Pro-443 to Gly-454.
888402	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6272 as residues: Leu-11 to Asn-16, Gly-164 to Glu-171, Leu-181 to Ser-186, Asp-193 to Ser-201, Glu-222 to Leu-229, Gln-238 to Tyr-245, Leu-256 to Asp-267, Gly-286 to Gln-301, Ser-311 to Ala-319, Glu-345 to Gly-351, Phe-361 to Asp-367, Thr-436 to Arg-443, Ile-460 to Gln-467, Gln-510 to Glu-533, Ala-541 to Ala-548, Gln-561 to Glu-571, Leu-581 to Ala-590, Phe-639 to Ser-652.
888708	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6275 as residues: Ile-27 to Val-33, Ala-63 to Ser-69, Pro-128 to Ser-135.
888720	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6276 as residues: Phe-34 to Glu-44, Glu-111 to Gly-122.
888950	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6278 as residues: Lys-56 to Gln-64, Pro-172 to Gly-183, Asp-208 to Asn-216, Glu-227 to Gly-232, Pro-259 to Arg-269, Asn-281 to His-286.
889136	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6279 as residues: Arg-1 to Lys-14, Glu-19 to His-26.
889263	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6280 as residues: Gly-18 to Gly-30.
889299	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6281 as residues: Leu-5 to Ser-12.
889300	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6282 as residues: Glu-15 to Gly-22, Asn-45 to Pro-51, Glu-141 to Asn-146, Asp-154 to Gln-163, Glu-185 to Ser-191, Arg-200 to Pro-206, Asp-220 to Asn-225, Glu-231 to Asn-237, Ser-262 to Gly-269, Pro-276 to Ala-281, Glu-314 to Thr-320, Ser-416 to His-424, Gly-426 to

	Ala-438, Pro-445 to Phe-450, Arg-464 to Leu-469.
889323	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6283 as residues: Pro-1 to Gly-11, Pro-13 to His-42, Arg-55 to Arg-66, Arg-84 to Gly-91, Gly-96 to Pro-101, His-112 to Pro-118.
889368	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6284 as residues: Pro-1 to Asn-9.
889467	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6285 as residues: Asp-10 to Asp-19, Ala-63 to Asp-68.
889494	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6286 as residues: Arg-1 to Ser-6.
889700	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6287 as residues: Ala-4 to Gly-14, Pro-20 to Cys-27, Leu-88 to Gly-94, Gly-106 to Lys-120, Pro-144 to Leu-150.
889782	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6288 as residues: Val-103 to Ser-108.
889954	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6289 as residues: Glu-21 to Tyr-33, Ile-90 to Ser-95, Pro-103 to Val-111, Ala-133 to His-140, Asn-153 to Trp-159, Gln-187 to Glu-192, Lys-214 to Arg-224.
889994	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6291 as residues: Ala-1 to Gln-7, Lys-24 to Ser-30, Pro-44 to Ser-49.
890666	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6292 as residues: Pro-36 to Trp-51, Arg-96 to Gly-104, Glu-134 to Asn-144, Pro-203 to His-210, Cys-228 to Asp-235, Gly-278 to Tyr-284, Ser-309 to Pro-316, Thr-325 to Ala-333, Ser-337 to Glu-357, Tyr-390 to Gly-403, Tyr-409 to Gly-421.
890698	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6293 as residues: Ser-37 to Asp-43.
890776	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6296 as residues: Ser-4 to Trp-13, Pro-276 to Ala-282, Ala-341 to Arg-347.
890801	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6297 as residues: Asn-9 to Arg-15.
890820	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6298 as residues: Arg-110 to Asp-115, Leu-185 to Gln-193, Ser-201 to Asp-208, Arg-215 to Arg-221, Arg-242 to Tyr-250, Thr-315 to Thr-320, Lys-359 to Val-367, Ser-395 to Tyr-401, Met-406 to Lys-411.
891264	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6302 as residues: Asp-1 to Gly-15, Ala-22 to Tyr-28.
891305	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6303 as residues: Asp-39 to Tyr-44, Thr-46 to Asn-55, Ser-78 to Ala-87.
892113	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6305 as residues: Gln-15 to Gln-22, Leu-216 to Lys-223.
892177	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6306 as residues: His-8 to Gly-18, Glu-100 to Asn-107, Glu-121 to Asn-126, Lys-128 to Ala-140, Ala-180 to Arg-186, Phe-230 to Thr-238, Pro-325 to His-341.

892367	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6308 as residues: Ser-31 to Gln-40..
892563	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6310 as residues: Arg-1 to Gly-23.
892820	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6311 as residues: Pro-8 to Thr-19.
893457	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6313 as residues: Lys-12 to Thr-18.
893827	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6314 as residues: Glu-37 to Asn-42, Ser-48 to Thr-54, Pro-101 to Glu-106.
893842	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6315 as residues: Asp-1 to Tyr-7, His-71 to Pro-78.
893866	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6316 as residues: Ala-12 to Lys-28, Ala-88 to Gly-95, Thr-100 to Cys-109.
894012	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6318 as residues: Ser-39 to Gln-48, Ala-61 to Pro-69.
894051	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6319 as residues: Arg-52 to Glu-66.
894121	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6320 as residues: Gly-28 to Ser-36, Trp-38 to Pro-60, Pro-98 to Thr-104, Pro-113 to Tyr-118, Phe-133 to Gly-140, Pro-186 to Leu-192, Glu-239 to Gly-246, Pro-257 to Lys-269, Lys-273 to Lys-279.
894341	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6321 as residues: Asn-18 to Asp-29.
894631	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6323 as residues: Met-1 to Gly-17, Pro-22 to Gly-30, Gly-72 to His-82, Leu-89 to Lys-95.
894806	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6324 as residues: Leu-99 to Ser-104.
894811	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6325 as residues: Asn-1 to Asn-8, Phe-49 to Asn-54, Glu-57 to Ser-63.
894820	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6327 as residues: Leu-8 to Gly-15.
894824	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6328 as residues: Ser-8 to Asp-13, Arg-19 to Ser-25.
894827	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6329 as residues: Arg-5 to Lys-11.
894830	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6330 as residues: Thr-102 to Gln-132.
894831	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6331 as residues: Ile-132 to Gly-138, Phe-149 to Thr-154.
894832	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6332 as residues: Pro-6 to Lys-17, Ser-66 to Pro-72, Pro-84 to Val-93.
894842	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6333 as residues: Ser-65 to Asp-70.

894878	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6334 as residues: Arg-9 to Trp-27, Pro-39 to Asn-44.
895122	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6335 as residues: Thr-11 to Pro-34, Asn-151 to Glu-157, Asp-302 to Phe-309, Tyr-333 to Gly-339.
895303	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6336 as residues: His-1 to Asp-9, Leu-11 to Glu-24, Pro-59 to Gln-65.
895372	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6337 as residues: Asn-7 to Ser-19, Arg-81 to Asn-94, Lys-99 to Asp-104.
895675	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6338 as residues: Asn-47 to Gly-52, Pro-67 to Asp-72, Pro-100 to Leu-105, Ser-115 to Asp-120, Leu-128 to Asn-135.
895927	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6340 as residues: Asn-3 to Trp-18, Gly-30 to Ser-35, Pro-41 to Ser-51, Ser-132 to Tyr-143.
896008	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6341 as residues: Pro-5 to Thr-28, Val-65 to Gly-71, Thr-82 to Gly-96.
897234	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6342 as residues: Ala-1 to Asp-10, Leu-24 to Phe-30, Pro-36 to Ser-42.
897524	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6343 as residues: Thr-1 to Cys-24, Lys-26 to Ser-32, Gln-83 to Thr-91, Thr-131 to Gly-137, Lys-170 to Asp-177, Asp-190 to Pro-198.
897898	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6344 as residues: Pro-23 to Arg-31, Gln-79 to Gln-85, Cys-93 to Cys-107.
898087	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6345 as residues: Ser-49 to Asp-59, Arg-69 to Tyr-87.
898136	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6346 as residues: Ser-12 to Ser-19, Ala-47 to Lys-52, Arg-96 to His-105.
898192	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6348 as residues: His-9 to Ile-14, Tyr-58 to Phe-64, Thr-75 to Phe-81.
898355	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6349 as residues: Pro-5 to Gly-18, Pro-21 to Asn-31, Gln-38 to Glu-43, Arg-63 to Arg-78.
898427	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6351 as residues: Gly-6 to Ile-11, Pro-13 to Arg-38, Glu-68 to Lys-74, Asp-88 to Ser-93, Glu-122 to Gly-130, Glu-145 to Glu-150, Thr-156 to Asp-174, Glu-200 to Arg-208, Ala-226 to Leu-240.
898541	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6352 as residues: His-1 to Leu-11, Arg-37 to Ile-43, Gln-111 to Pro-120, Asp-133 to Asn-138, Arg-159 to Cys-165, Val-241 to Lys-265, Glu-326 to Tyr-331, Pro-365 to Asn-382, Asn-418 to Asp-430, Ala-434 to Ser-441, Tyr-479 to Gly-496, Pro-498 to Ser-505.
898651	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 6353 as residues: Ser-6 to Pro-11, Pro-27 to Glu-32, Pro-65 to Trp-71, Val-208 to Pro-215, His-220 to Thr-225.
898946	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6355 as residues: Thr-4 to Arg-14, Glu-34 to Pro-46.
899130	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6356 as residues: Pro-10 to His-19, Leu-47 to Tyr-55, Phe-93 to Gly-105, Ser-220 to Trp-227, Phe-295 to Thr-301, Thr-309 to Trp-315, Arg-326 to Phe-334, Arg-458 to Pro-466.
899224	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6357 as residues: Ser-3 to Gly-28, Gly-46 to Pro-56, Gly-70 to Ile-92, Gln-102 to Ser-117, Ala-123 to Pro-129, Pro-135 to Leu-140, Pro-150 to Asp-158, Pro-165 to Pro-177, Gln-188 to Asp-205, Ile-230 to Arg-245, His-251 to Trp-260, Asp-262 to Cys-267, Asn-296 to Arg-307, Glu-322 to Pro-330, Ile-351 to Asn-357, Asp-363 to Leu-369, Glu-386 to Phe-391, Lys-415 to Ser-420.
899632	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6358 as residues: Thr-11 to Ser-16, Gly-25 to Asn-40.
899661	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6360 as residues: His-8 to Gly-18, Pro-35 to Trp-41, Arg-51 to Asp-64, Asp-69 to Gln-74, Gly-83 to Asn-96, Pro-107 to Lys-116, Glu-149 to Ser-171, Ile-177 to Ile-186.
899776	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6361 as residues: Met-36 to Arg-49, Pro-72 to Gly-82, Glu-89 to Gly-96, Tyr-129 to Thr-135.
899885	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6363 as residues: His-65 to Gly-74, Asp-85 to Ser-97, Leu-133 to Glu-138, Glu-144 to Asp-153, Arg-170 to Ser-175, Gly-184 to Arg-189, Gln-202 to Tyr-208.
899913	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6364 as residues: Lys-1 to Tyr-16.
900015	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6365 as residues: Lys-23 to His-36, Asp-52 to Leu-68.
900162	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6366 as residues: Gly-1 to Leu-9, Gly-48 to Gln-53, Cys-74 to Pro-79, Thr-118 to Val-128.
900555	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6368 as residues: His-8 to Gly-18, Cys-131 to Gly-136, Thr-198 to Asn-203, Pro-231 to Asp-236.
900696	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6369 as residues: Arg-11 to Ser-23, Arg-72 to Pro-84, Asp-90 to Ser-103, Gly-172 to Glu-179, Pro-190 to Phe-197, Val-210 to Arg-216, Pro-228 to Leu-233.
900777	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6370 as residues: Pro-5 to Arg-16, Thr-21 to Gly-27, Ser-35 to Gln-40, Arg-103 to Lys-112, Gly-172 to Pro-188, Gln-190 to Met-198.
900784	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6371 as residues: Gln-36 to Trp-52, Gly-164 to Gly-175, Ile-210 to Arg-215, Asn-417 to Val-422, Val-426 to Gln-431, Val-439 to Gly-444, Lys-470 to Leu-481, Phe-500 to Ser-511, Met-553 to Gly-563, Glu-691 to Thr-700, Ile-714 to Gly-723, Ala-750 to Gly-762, Leu-788 to

	Phe-794, Ser-798 to Gln-803, Thr-811 to Lys-816, Ser-824 to Phe-835, Thr-882 to Glu-892, Leu-901 to Gln-907, Gln-937 to Met-944.
900838	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6372 as residues: Pro-9 to Gly-15, Pro-47 to Pro-69, Pro-113 to Cys-122.
900966	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6374 as residues: Arg-34 to Gly-42, Gly-53 to Ser-59, Ala-74 to Gly-81, Glu-89 to Gly-103, Gly-108 to Gly-113, His-120 to Gly-223, Asp-225 to Gly-243, Pro-247 to Gly-312, Gly-317 to Asp-322.
901111	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6377 as residues: Pro-17 to Asp-36, Pro-102 to Glu-108, Pro-122 to Lys-128, His-150 to Gly-155, Asn-162 to Tyr-168, Pro-186 to Gln-193, Ser-205 to Pro-211, Gln-305 to Gly-317.
901128	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6379 as residues: Pro-1 to Gly-8, Pro-38 to Pro-45, Thr-103 to Ser-109, Cys-112 to Trp-119, Ala-201 to His-210, Glu-230 to Asn-241, Trp-263 to Ala-269.
901202	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6380 as residues: Pro-1 to Leu-17, Gly-36 to Gly-49.
901253	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6381 as residues: Gly-13 to Met-26, Arg-34 to Gly-39, Ile-60 to Ser-80, Ala-85 to Thr-98.
901276	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6382 as residues: Gln-1 to Arg-24, Gln-41 to Ala-48, Ser-70 to Gly-82, Glu-104 to Phe-112, Lys-126 to Ser-132, Pro-276 to Ile-281.
901333	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6383 as residues: Gln-48 to Lys-64, Glu-175 to Thr-183.
901375	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6384 as residues: Pro-3 to Lys-8, Phe-43 to Gly-51, Lys-55 to Ala-62, Ser-92 to Gln-98, Asp-106 to Trp-113, Ser-125 to Asn-134, Ser-150 to Phe-160.
901421	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6386 as residues: Arg-29 to Leu-38, Lys-47 to Arg-53, Asp-70 to Thr-75, Glu-116 to Leu-124, Gln-134 to Ser-143, Ser-158 to Trp-163, Pro-168 to Asp-180.
901472	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6387 as residues: Arg-1 to Val-7, Ala-156 to Phe-162.
901473	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6388 as residues: Leu-39 to Ile-47, Val-92 to Arg-98, Tyr-146 to Leu-160, Asp-185 to Phe-192, Phe-195 to Gly-207.
901494	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6389 as residues: Pro-11 to Trp-16, Gln-25 to Ser-37, Pro-99 to Gly-104, Pro-109 to Gly-115, Trp-201 to Thr-209.
901515	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6390 as residues: Gln-46 to Leu-51, Asp-58 to Asn-65, Lys-70 to Gln-75, Pro-111 to Thr-117, Gly-176 to Gly-185, Asp-205 to Gly-213, Thr-247 to Ile-263, Leu-269 to Lys-279.
901567	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6391 as residues: Phe-3 to Ala-8, Pro-17 to Gly-24, Asn-162 to Gln-179, Asn-195 to Asp-201, Glu-207 to Leu-213.

901578	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6392 as residues: Leu-1 to Glu-13, Ile-34 to Arg-40, Lys-46 to Arg-57, Ala-77 to Ile-88, Pro-103 to Asp-111, Phe-127 to Ser-138.
901621	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6393 as residues: Gln-7 to Gly-12, Leu-60 to Pro-65, Arg-85 to Lys-99, Ser-132 to Pro-145, Pro-150 to Asp-155, Pro-183 to Asn-193, Arg-200 to Tyr-206.
901875	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6394 as residues: Gly-13 to Met-26, Arg-34 to Gly-39, Ile-60 to Ser-80, Ala-85 to Thr-98, Asn-109 to Val-140, Lys-150 to Thr-157, Gly-174 to Ala-201, Thr-204 to Lys-212, Thr-237 to Gly-243, Pro-251 to Pro-261, Ala-263 to Lys-277, Phe-281 to Arg-286, Arg-333 to Asp-341, Glu-407 to Asp-412, Gly-424 to Gly-430, Gly-570 to Trp-583, Gln-614 to Gly-619.
HCRMU56 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6396 as residues: Leu-7 to Leu-13, Pro-15 to Gln-27.
HKCSA70R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6398 as residues: Leu-29 to Val-34, Gln-42 to Gly-52.
HWLOB10 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6399 as residues: Gly-49 to Pro-54.
HCQCG26R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6400 as residues: Gly-1 to Asp-6, Asp-16 to Ser-21, Val-36 to Cys-43, Ser-51 to Leu-60, Ile-65 to Lys-70.
HOENF69R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6404 as residues: Ala-15 to Ser-32, Ser-34 to Gly-43, Thr-57 to Gly-65.
HWLQY33 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6406 as residues: Gln-17 to Lys-22.
HCRNF08R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6407 as residues: Arg-1 to Arg-13, Asn-33 to Arg-39.
HKCSZ69R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6408 as residues: Thr-32 to Lys-37.
HCQAG23 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6409 as residues: Arg-22 to Thr-28.
H2LAF75R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6411 as residues: Gly-1 to Ser-6.
H2LAT73R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6413 as residues: Thr-3 to Ser-10.
HUUAQ45 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6422 as residues: Arg-13 to Asn-22, Lys-42 to Glu-48.
HWLWQ51 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6423 as residues: Ala-18 to Asn-24, Thr-65 to Arg-71, Val-84 to Thr-96.
HKLAB44R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6424 as residues: Val-7 to Trp-19, Ser-73 to Ser-79, Lys-86 to Ser-94.
H2CBA06R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6425 as residues: Ala-12 to Asp-20, Glu-30 to Arg-40, Gln-51 to Arg-57, Arg-79 to Tyr-88.

HCNAH60 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6427 as residues: Arg-19 to Gly-32.
HWMBJ68 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6432 as residues: Glu-10 to Gly-16, Asp-62 to Arg-69.
HELGR96R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6435 as residues: Leu-31 to Gln-39.
HCRQM72 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6437 as residues: Asn-5 to Lys-14, Glu-25 to Gly-33, Arg-48 to Thr-74.
HWLMH52 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6440 as residues: Glu-24 to Leu-30.
H2CBU03R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6441 as residues: Thr-2 to Ser-9.
HCQDR91R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6443 as residues: Gly-14 to Arg-19.
HWMBN34 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6444 as residues: Lys-7 to Thr-12, Pro-25 to Lys-30, Leu-38 to Asp-43, Ser-84 to Ala-95, Asp-108 to Ser-117.
HCRNF81R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6447 as residues: Pro-12 to His-17, Gln-57 to Asp-62, Thr-79 to Lys-101, Thr-117 to Ser-129.
HOHCI31R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6448 as residues: Leu-16 to Ser-22, Lys-24 to Glu-38.
HSKKC10R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6449 as residues: Glu-4 to Gly-10.
H2CBC52R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6452 as residues: Pro-18 to Ser-30, Pro-37 to Pro-43.
HWLMC24 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6454 as residues: Pro-4 to Gly-34.
HWLUR40 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6455 as residues: Phe-3 to Lys-12.
HHAOD46 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6457 as residues: Lys-23 to Ala-40, Pro-67 to Ala-72, Val-102 to Thr-110.
HCYBA83R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6458 as residues: Trp-13 to Ile-21, Pro-59 to Thr-68, Ala-85 to Lys-92, Thr-102 to Gly-113.
HCROZ77R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6459 as residues: Asp-1 to Arg-8, Lys-13 to Leu-18, Gly-32 to Glu-49, Lys-60 to Ala-75, Ser-84 to Asp-99, Glu-107 to Ser-119, Ala-132 to Gly-141.
HCQCP20R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6461 as residues: Leu-18 to Gln-25, Lys-37 to Phe-45.
HWLNF84 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6463 as residues: Lys-17 to Asn-22, Glu-31 to Lys-36, Gln-38 to Arg-44, Thr-81 to Thr-88.
HCRQI10R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6468 as residues: Asp-56 to Lys-63, Lys-78 to Asn-86, Phe-92 to Lys-99.

HULCD94R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6470 as residues: Lys-7 to Thr-13, Asp-24 to Thr-30, Gly-39 to Glu-52, Leu-70 to Arg-76, Phe-87 to Tyr-92.
HHMMF84 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6471 as residues: Lys-30 to His-37.
HCRPO08R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6472 as residues: Val-33 to Lys-38.
HWLMQ74 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6475 as residues: Pro-9 to Gly-21.
H2LAB80R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6478 as residues: Thr-14 to Val-32.
HCQDO33 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6480 as residues: Trp-10 to Gly-18, Arg-34 to Pro-39.
HKAFL06R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6482 as residues: Pro-1 to Gly-14, Cys-18 to Gly-24, Ala-39 to Arg-55, Gly-63 to Glu-76, Gln-106 to Arg-115.
HWLOO35 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6486 as residues: Gly-1 to Gly-7, Arg-13 to Glu-19.
HWLVL77 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6487 as residues: Arg-13 to Gly-40.
HBJMG15R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6489 as residues: Ser-14 to Glu-27, Ile-40 to Ile-54.
H2CBH29R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6490 as residues: Ser-16 to Glu-21.
H2LBB21R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6494 as residues: Phe-50 to Tyr-55, Thr-63 to Trp-69, Pro-74 to Arg-80.
H2LAT69R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6495 as residues: Thr-2 to Ser-11.
HLWCJ40R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6496 as residues: Tyr-28 to Pro-40.
HOGDQ57 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6498 as residues: Pro-1 to Gln-8, Met-20 to Leu-26, Gly-42 to Ser-49, Ile-63 to Pro-73, Gly-80 to Ala-87.
HWLQM12 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6499 as residues: Pro-45 to Gly-52.
H2CBG89R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6501 as residues: Met-2 to Asp-31, Leu-67 to Asp-74, Gly-93 to Ser-98.
HWLWQ68 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6502 as residues: Ser-21 to Glu-38.
HCYBM79 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6503 as residues: Glu-11 to Lys-22, Asp-31 to Trp-50.
HMUBO53 RA	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6504 as residues: Glu-1 to Asp-6, Asn-92 to Leu-97.
HWLVN81 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6506 as residues: Arg-6 to Val-14.
HWLRV71 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6507 as residues: Asp-34 to Pro-45.

HDPMJ48R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6511 as residues: Thr-1 to Trp-14, Lys-27 to Leu-44, Glu-59 to Arg-73, Lys-87 to Phe-95.
HWLNJ72R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6512 as residues: Ala-21 to Pro-30, Thr-43 to Glu-51.
HOFME52 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6513 as residues: Pro-7 to Phe-14, Glu-22 to Lys-28, Ala-31 to Glu-39, Lys-47 to Asp-54.
HCRMG55 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6515 as residues: Pro-4 to Gly-10, Lys-28 to Thr-37, Glu-45 to Glu-55.
HCRNZ49R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6516 as residues: Pro-1 to Ala-14.
H2LAD43R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6518 as residues: Gly-1 to Ser-6, Pro-20 to Trp-31.
HCQCB53R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6522 as residues: Pro-8 to Asn-18.
HCQCL32R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6525 as residues: Arg-3 to Asn-18.
HCQCP47R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6526 as residues: Thr-4 to Ser-11.
HCQDC76R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6527 as residues: Asp-1 to Lys-6, Lys-11 to Ser-17.
HCQDH59 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6528 as residues: Gly-1 to Gly-8.
HCQDK53 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6531 as residues: Gly-1 to Gly-8.
HCQDP62R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6535 as residues: Gly-1 to Gly-8.
HKCAA76 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6543 as residues: Ser-31 to Tyr-36, Pro-64 to Gly-72.
HCRNF45R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6546 as residues: Pro-8 to Glu-13, Pro-27 to Pro-33.
HCROB90R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6555 as residues: Arg-63 to Gly-69.
HCRNI50R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6556 as residues: Ser-15 to Ile-24, Asn-56 to Lys-67, Ser-80 to Lys-95.
HCRPJ34R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6557 as residues: Val-5 to Gln-11.
HCQBL95R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6558 as residues: Ser-4 to Pro-10, Glu-18 to Cys-23.
HWLOR95 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6559 as residues: Ser-23 to Ala-28, Pro-64 to Glu-74, Ala-100 to Lys-106.
HKCSI32R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6560 as residues: Ala-4 to Gln-14, Gly-36 to Gln-42, Gly-70 to Leu-77.
HBCJN86R	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 6565 as residues: Pro-6 to Tyr-17, Val-39 to Gln-45.
HWLMZ47 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6567 as residues: Ile-45 to Gly-50.
HCRPD88R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6569 as residues: Asn-15 to Phe-27, His-39 to Ser-44, Glu-49 to Ala-55.
HCQDC47R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6571 as residues: Asp-1 to Asn-7, Pro-22 to Ser-28, Leu-54 to Asn-59, Gly-95 to Arg-101.
H2CBR33R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6573 as residues: Ile-2 to Leu-8.
HWLXV36 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6574 as residues: Lys-14 to Gln-24, Pro-32 to Ile-40.
HWLRE24 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6575 as residues: Lys-20 to Gly-38, Val-42 to Thr-53, Ala-88 to Ala-99.
HWMB A27 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6576 as residues: Gly-35 to Glu-62.
HWMBK08 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6577 as residues: Asp-2 to Cys-8.
HCQCT96R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6582 as residues: Pro-1 to Glu-14.
HWLXR95 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6584 as residues: Lys-22 to Ser-33, Ala-39 to Glu-48, Lys-70 to Lys-75.
HEPAD45R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6586 as residues: Met-42 to Arg-53.
HCRNP41R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6587 as residues: Arg-25 to Asn-34, Lys-54 to Glu-60.
HCYBK83 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6588 as residues: Pro-1 to Ser-6.
HCRND59R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6589 as residues: Phe-88 to Pro-93, Thr-102 to Pro-113.
HCRMA15 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6592 as residues: Gly-4 to Lys-10, Gln-36 to Glu-41.
HCRMJ42R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6593 as residues: Gly-4 to Lys-10, Gln-36 to Glu-41.
HCRMO88 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6595 as residues: Gly-4 to Lys-10, Gln-36 to Glu-41, Phe-57 to Asn-62.
HCRNB87R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6596 as residues: Arg-17 to His-22.
HCRNL44R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6598 as residues: Ser-2 to Ala-7.
HCRPK46R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6603 as residues: Tyr-3 to Gly-10, Ala-17 to Tyr-24.
HCRPK48R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6604 as residues: Asn-1 to Arg-9.
HCRQG02R	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 6606 as residues: Tyr-1 to Gly-14.
HCRQM26 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6607 as residues: Tyr-1 to Gly-16.
HHMMA34 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6609 as residues: Gly-4 to Leu-11.
HHMMA44 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6610 as residues: Gly-4 to Lys-10, Gln-36 to Glu-41.
HHMMC42 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6611 as residues: Gly-4 to Lys-10.
HHMMC86 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6612 as residues: Gly-4 to Lys-10, Gln-36 to Pro-43.
HHMME38 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6614 as residues: Gly-4 to Lys-10, Gln-36 to Lys-43.
HHMME80 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6618 as residues: Gly-4 to Lys-10, Gln-36 to Lys-43.
HHMMF79 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6620 as residues: Val-2 to Gly-9.
HOCTA39R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6621 as residues: Lys-7 to Lys-19.
HULCG37R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6623 as residues: Ile-2 to Ser-15, Gln-30 to Asp-38.
HWLMQ27 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6629 as residues: Pro-16 to Tyr-23.
HWLMQ65 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6632 as residues: Gln-37 to Arg-42.
HWLNZ20 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6634 as residues: Pro-12 to Glu-21.
HWLNZ35 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6635 as residues: Pro-16 to Gly-35.
HWLNZ44 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6636 as residues: Pro-13 to Glu-22.
HWLOW58 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6639 as residues: Gly-4 to Lys-10.
HWMB518 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6653 as residues: Pro-10 to Trp-21.
HCRPY45R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6662 as residues: Lys-7 to Lys-20, Gln-46 to Glu-51.
HHMMF44 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6667 as residues: Gly-8 to Leu-15, Gln-40 to Lys-48.
HTWEL13 RA	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6668 as residues: Cys-6 to Ser-12.
HCRMH46 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6674 as residues: Gln-19 to Glu-24.
HWLND45 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6676 as residues: Gly-4 to Lys-11.
HWLWG95 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6677 as residues: Arg-21 to Arg-36.
HCRQO33R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6679 as residues: Pro-6 to Asp-21.

HCRMJ70R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6682 as residues: Leu-18 to Asp-41.
HWLMM72 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6683 as residues: Asp-42 to Asn-47.
HCRMD32 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6684 as residues: Ala-17 to Lys-28, Glu-51 to Gln-56, Ser-64 to Lys-72.
HKAHM80 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6688 as residues: Lys-10 to Ala-17, Glu-27 to Leu-37, Met-74 to Lys-80, Pro-94 to Gln-108.
H2CBM60R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6689 as residues: His-23 to Arg-30, Asp-61 to Asn-73, Phe-89 to Gln-97.
HWLXR73 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6692 as residues: Arg-1 to Pro-11, Gly-16 to Gly-21, Gly-28 to Gly-43.
HWLOI59R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6693 as residues: Val-9 to Leu-20, Lys-44 to Pro-51.
HWLUX53 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6694 as residues: Gly-1 to Glu-10.
HARMO20 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6696 as residues: Arg-2 to Val-18.
HCQDM81 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6699 as residues: Arg-2 to Val-18.
HFJJB15R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6703 as residues: Pro-26 to Gln-32.
HACCH14R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6704 as residues: Thr-1 to Tyr-7.
HCRPV08R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6708 as residues: Val-26 to Val-33, Phe-41 to Ser-55, Val-62 to Gly-72.
HWMBB77 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6709 as residues: Gln-7 to Leu-17, Lys-110 to Cys-116, Asn-133 to Asn-138.
HHEPL48R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6710 as residues: Thr-2 to Met-11, Cys-15 to Pro-20, Asp-28 to Ser-33, Lys-40 to Gly-45.
HCRPT53R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6711 as residues: Tyr-9 to Phe-14, Glu-30 to Lys-39.
HTXJU67R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6714 as residues: Ile-10 to Gln-15, Pro-22 to Asn-28.
HWMCCL33 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6715 as residues: Ala-83 to Ala-88.
HCQCO67R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6717 as residues: Cys-15 to Ser-30, Ser-39 to Met-45.
HWLVI33R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6719 as residues: Arg-32 to Gly-38.
HWMBAA55 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6720 as residues: Thr-1 to Gln-7, Thr-26 to Leu-36, Ala-86 to

	Asp-104, Ser-114 to Val-121.
HCRON89R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6725 as residues: Ala-15 to Gly-22.
HLDDP53R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6727 as residues: Ala-25 to Asp-32.
HWLME23 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6728 as residues: Ala-9 to Arg-15.
HWLVP88 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6729 as residues: Arg-21 to Ser-28, Gly-115 to Gln-142.
HWLMG29 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6739 as residues: Ser-16 to Lys-21, Pro-34 to Lys-41.
HCQCF55R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6740 as residues: Arg-1 to Arg-26, Ser-42 to Tyr-50, Glu-60 to Cys-69.
HWLWB88 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6742 as residues: Pro-6 to Glu-13.
HWLXR58 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6747 as residues: Glu-4 to Asp-12, Glu-19 to Lys-29, Ser-32 to Glu-40, Glu-51 to Thr-56, Ile-58 to Ser-79, Ser-86 to Glu-95.
HCYBO60R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6750 as residues: His-8 to Gly-18, Gly-26 to Pro-35, Pro-58 to Asp-64.
HE2BG62R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6751 as residues: Phe-10 to Tyr-15.
HCRMW12 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6752 as residues: Gly-21 to Asn-31, Cys-62 to Lys-68, Pro-76 to Thr-81, Cys-105 to Arg-124, Lys-139 to Gln-145, Gly-151 to Gly-158.
HWLVF61 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6755 as residues: Tyr-12 to Ile-17, Pro-28 to Asn-33, Arg-45 to Asp-53.
HWMBP47 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6757 as residues: Val-1 to Val-10.
HWLQF89 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6760 as residues: Pro-8 to Pro-25, Asp-72 to Thr-78, Glu-81 to Ser-87.
HWMCC54 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6763 as residues: Gln-66 to Ser-71, Ser-80 to Gly-92.
HCQAS76R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6767 as residues: Thr-34 to Ser-40.
HKLRA71R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6768 as residues: Ile-1 to Ser-9.
HWMCJ58 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6769 as residues: Pro-10 to Arg-18.
HWLMJ20 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6770 as residues: Pro-56 to Trp-61.
HWLMU79 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6773 as residues: Trp-4 to Lys-11.
HWLNN06	Preferred epitopes include those comprising a sequence shown in SEQ

R	ID NO. 6775 as residues: Gln-27 to Ser-32, Trp-57 to Ser-65, Glu-72 to Ser-85, Lys-103 to Ser-117.
HWLMM42 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6776 as residues: Asn-43 to His-64.
HWMBBC38 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6777 as residues: His-61 to Gly-68.
HWLVU11 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6778 as residues: Val-65 to Thr-74, Ser-84 to Asn-101.
HCQDW90 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6785 as residues: Arg-18 to Ser-24.
HCYBM34 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6786 as residues: Arg-22 to Ser-28.
HCYBM57 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6787 as residues: Arg-31 to Thr-38.
HCQCK49 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6789 as residues: Phe-14 to Ser-22.
HWLRQ41 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6792 as residues: Lys-13 to Asp-24.
HWLOC77 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6793 as residues: Phe-47 to Ser-52.
HDDNQ21 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6794 as residues: Tyr-6 to Gly-13, Asn-35 to Thr-42, Pro-47 to Glu-56.
HCQDA89 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6796 as residues: Leu-7 to Arg-13.
HCQCO43R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6797 as residues: Asp-18 to Arg-29.
HCQCG73R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6798 as residues: Lys-12 to Asn-18, Glu-24 to Glu-31, Ile-40 to Ala-53, Pro-65 to Asp-75.
HWLQA92 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6799 as residues: Arg-10 to Ser-18, Pro-27 to Lys-36.
HCROM41 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6802 as residues: Ser-1 to Arg-9, Thr-40 to Trp-47, Ser-84 to Asp-95, Leu-113 to Asn-127, Pro-140 to Arg-151.
H2LAA02R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6803 as residues: Ala-11 to Pro-20, Asn-39 to Val-46.
HCQDU29 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6805 as residues: Val-1 to Met-8.
HWMBJ73 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6811 as residues: Arg-41 to Glu-46.
HCRNO44R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6814 as residues: Lys-1 to Thr-6.
HSAMD89 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6818 as residues: Leu-32 to Glu-59, Lys-67 to Lys-89.
HCROE42R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6820 as residues: Phe-48 to Gly-56, Ile-60 to Glu-65, Pro-73 to Trp-80, Ser-100 to Lys-117, Lys-126 to Ser-138.
HCROE77R	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 6824 as residues: Asp-46 to Lys-51.
HOCTA19R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6827 as residues: Ser-3 to Ala-12, Gly-71 to Val-84.
HWLOM88 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6829 as residues: Glu-11 to Cys-17, Ala-26 to Trp-31, Ser-43 to Glu-55, Gly-127 to Ala-132.
H2CBI14R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6830 as residues: Lys-21 to Lys-29.
HCRNI08R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6831 as residues: Glu-10 to Val-16, Thr-59 to Ser-66, Asp-112 to Ala-121, Pro-147 to Ala-157.
HFPBS29R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6832 as residues: Pro-22 to His-30.
HCQCB43R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6834 as residues: Asn-4 to Tyr-9.
HCQDB27R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6836 as residues: Asn-4 to Tyr-9.
HCQCR82R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6838 as residues: Glu-9 to Gly-17.
HWLWH33 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6842 as residues: Arg-10 to Arg-15, Val-25 to Gly-33, Pro-45 to Asp-51.
HCYBJ83R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6843 as residues: Arg-1 to Gly-6, Arg-60 to Gly-65.
HWLRE17 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6844 as residues: Gln-34 to Gly-46, Gly-54 to Arg-61, Pro-67 to Gly-82, Glu-91 to Asn-114.
HWLOM10 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6846 as residues: Glu-1 to Arg-11, Thr-18 to Ser-39, Ala-51 to Leu-56, Pro-69 to Gly-78, Glu-88 to Ala-93, Pro-114 to Lys-126, Leu-133 to Thr-141.
H2LBA48R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6848 as residues: Thr-13 to Thr-23.
HCRPZ16R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6849 as residues: Ala-14 to Cys-32, Lys-34 to Arg-40, Ser-46 to Trp-52, Arg-59 to Gly-64.
HKCSA80R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6850 as residues: Asn-39 to Gln-44.
HCRPH64R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6852 as residues: Arg-38 to Ser-46.
HDTBZ03R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6853 as residues: Lys-1 to Gly-28, Thr-50 to Leu-57, Glu-70 to Trp-90, Pro-93 to Asp-100.
HLYED39R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6854 as residues: Arg-2 to Thr-9.
HCQCB85R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6855 as residues: Gly-9 to Ser-14, Gln-26 to Gly-37.
HCRNF48R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6858 as residues: Glu-29 to Leu-34, Thr-40 to Pro-45, Ser-68 to

	Met-73.
HWLQA11 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6860 as residues: His-60 to Cys-69.
HWLXJ34R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6863 as residues: Arg-13 to Leu-22, Ser-25 to Glu-30, Leu-32 to Ala-43, Thr-49 to Pro-55, Ala-69 to Tyr-76, Pro-83 to Ser-91, Glu-104 to Ser-115.
HCRQN67R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6865 as residues: Lys-1 to Ser-12, Arg-20 to Gln-25, Pro-80 to Arg-86.
HCYBH30R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6866 as residues: Thr-19 to Lys-27.
HCROE26R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6869 as residues: Arg-25 to Val-33, Ser-43 to Gly-48, Ala-54 to Gly-59.
HOHBE57R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6870 as residues: Asp-1 to Gln-14, Thr-34 to Pro-40, Asn-42 to Asp-57, Ala-112 to Gly-117.
HWMBB94 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6872 as residues: Ser-53 to Val-62.
HUVHA17 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6873 as residues: Glu-34 to Thr-41.
HLTIJ91R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6874 as residues: Glu-11 to Leu-21, Glu-42 to Gln-50.
HCRMC40 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6875 as residues: Arg-37 to Val-48.
HWLQD31 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6878 as residues: Ala-37 to Lys-42, Pro-55 to Asp-62.
HOSBE19R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6880 as residues: Asp-25 to Ile-31.
HWLQG37 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6882 as residues: Ala-10 to Lys-16, Lys-19 to Val-27.
HSAMB82 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6883 as residues: Gln-1 to Arg-11.
HWLWE05 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6884 as residues: Thr-5 to Thr-14.
HFVKA92R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6893 as residues: Asp-28 to Arg-34.
HKLSA82R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6894 as residues: Phe-1 to Glu-12, Gln-21 to Asp-28, Asp-30 to Pro-35.
HWLNK27 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6896 as residues: Gln-2 to Trp-8.
HCRNT24R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6897 as residues: Arg-13 to Thr-21, Ser-43 to Ala-49.
HCQAW95 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6898 as residues: Thr-2 to Lys-7, Lys-12 to Pro-21.
HFCESS3R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6902 as residues: Thr-12 to Leu-18.

HCQCQ84R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6903 as residues: Gly-1 to Ala-10.
HWMBC92 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6905 as residues: Leu-49 to Asn-62, Pro-65 to Leu-84.
HWLQQ35 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6908 as residues: Arg-6 to Ala-19, Asn-26 to Thr-50, Phe-57 to Ser-62, Asp-68 to Glu-96, Ser-102 to Gly-137.
HCRNZ02R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6911 as residues: Asn-1 to Lys-9, Cys-51 to Ala-65, Thr-74 to Arg-86.
HCQDW65 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6916 as residues: Lys-19 to Ser-27.
HCQDN27 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6918 as residues: Glu-6 to Gln-21.
HCQCI92R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6919 as residues: Pro-19 to Lys-40.
HCROT79R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6922 as residues: Gly-12 to Glu-18.
H2CAA07R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6923 as residues: Glu-8 to Ala-16, Tyr-25 to Trp-32.
H2LAD20R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6924 as residues: Ser-1 to Leu-6, Ser-22 to Leu-31.
HWLQZ32 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6925 as residues: Pro-1 to Leu-7, Gly-49 to Gly-69, Glu-100 to Ala-106.
HCRQK79 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6929 as residues: Lys-7 to Gly-14, Ala-31 to Gly-37.
HCQAD53 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6930 as residues: Thr-1 to Thr-13.
HKCUD58 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6931 as residues: Ser-21 to Cys-28.
HCRNR93R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6932 as residues: Lys-54 to Leu-64.
HWLQH13 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6933 as residues: Asp-12 to Ser-19, Leu-52 to Gln-57, Leu-79 to Glu-86, Asn-97 to Phe-109, Gln-134 to Asn-142, Arg-151 to Gly-156.
H2CBQ60R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6934 as residues: Ala-23 to Asp-32, Thr-42 to Gly-47, Pro-59 to Glu-67, Phe-77 to Ser-84.
H2LAW43 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6935 as residues: Thr-3 to Ser-12.
HWLVJ22R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6936 as residues: Gln-7 to Ser-23, Pro-63 to Lys-86.
H2CAA28R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6938 as residues: Glu-17 to Cys-22.
H2CAA36R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6939 as residues: Asp-1 to Arg-9.
H2CBG84R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6941 as residues: Gly-13 to Leu-20.

H2CBJ35R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6942 as residues: Val-3 to Ala-11, Ala-38 to Leu-51, Ser-53 to Pro-70, Gln-88 to Gly-94, Ser-106 to Ser-113.
H2CBK71R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6944 as residues: Pro-18 to Pro-24, Arg-31 to Thr-41.
H2CBN87R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6945 as residues: Asp-1 to Ser-6.
H2CBP73R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6946 as residues: Ala-2 to Ser-9, Pro-40 to Gly-54.
H2CBS94R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6947 as residues: Gly-39 to Gln-45.
H2CBV81R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6949 as residues: Arg-1 to Trp-8.
H2CBW73 RB	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6950 as residues: Trp-1 to Ser-8, Pro-17 to Glu-27, Gln-41 to Val-54, Asp-65 to Pro-76.
H2LAZ29R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6953 as residues: Asp-8 to Gly-18, Ala-21 to Arg-26, Glu-31 to Lys-36, Ser-61 to Gly-66.
H2LAZ92R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6954 as residues: His-10 to Phe-16, Thr-64 to Arg-79.
H2LBB20R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6956 as residues: Pro-17 to Arg-29, Gly-49 to Ala-62, Gly-70 to Lys-81.
HBAHC91R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6960 as residues: Gln-21 to Ala-27.
HCEOM04 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6962 as residues: Thr-2 to Lys-11.
HCFOE14R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6963 as residues: Glu-20 to Tyr-25, Phe-43 to Glu-48.
HCHOX67 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6964 as residues: Ser-16 to His-21, Ala-29 to Thr-35.
HCQAB27R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6965 as residues: Lys-1 to Val-13.
HCQAB44R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6968 as residues: Thr-19 to Thr-31.
HCQAB53R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6969 as residues: Ile-34 to His-39.
HCQAC03R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6970 as residues: Ser-51 to Gly-60.
HCQAD62 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6976 as residues: Ala-1 to Val-8, Arg-24 to Gly-36.
HCQAE39R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6981 as residues: Thr-3 to Arg-19.
HCQAG32 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6986 as residues: Arg-1 to Tyr-6.
HCQAI15R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6994 as residues: Gly-1 to Ala-8.
HCQAK16	Preferred epitopes include those comprising a sequence shown in SEQ

R	ID NO. 6998 as residues: Gly-1 to Ser-9.
HCQAK17 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 6999 as residues: Ala-1 to Arg-7.
HCQAL71R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7001 as residues: Val-2 to His-12.
HCQAM57 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7004 as residues: Arg-1 to Thr-8.
HCQAN95 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7012 as residues: Phe-11 to Ser-17, Leu-42 to Gly-47.
HCQAR63R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7016 as residues: Thr-5 to Arg-11.
HCQAS25R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7019 as residues: His-4 to His-10.
HCQAT12R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7025 as residues: Trp-2 to Gly-9.
HCQAV66 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7032 as residues: Gly-1 to Ser-8.
HCQAW40 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7036 as residues: His-1 to Ile-26, Leu-30 to Ser-37, Ala-59 to Leu-66.
HCQBA47R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7038 as residues: Ser-8 to Arg-14.
HCQBE19R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7043 as residues: Glu-25 to Ser-30.
HCQBL61R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7049 as residues: Arg-38 to Asn-43.
HCQBM58 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7052 as residues: Gln-7 to Glu-16.
HCQCC50R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7067 as residues: Arg-1 to Gly-8, Pro-11 to Asn-21, Gln-28 to Lys-36.
HCQCD10R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7072 as residues: Ser-33 to Tyr-42, Val-51 to Ser-56.
HCQCD46R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7073 as residues: Arg-14 to Thr-21.
HCQCE46R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7079 as residues: Ala-2 to Asp-10.
HCQCE83R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7085 as residues: Arg-14 to Thr-20.
HCQCF77R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7092 as residues: Lys-8 to Asn-19.
HCQCH16R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7104 as residues: Leu-31 to Thr-37, Gly-54 to Glu-61.
HCQCH47R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7108 as residues: Pro-13 to Glu-18.
HCQCJ42R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7126 as residues: Glu-1 to Gly-13.
HCQCJ51R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7129 as residues: Pro-8 to Asn-18, Gln-25 to Val-30.

HCQCJ77R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7132 as residues: Asn-1 to Thr-6.
HCQCJ89R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7134 as residues: Phe-16 to Asn-27.
HCQCK81R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7145 as residues: Glu-15 to Glu-20.
HCQCK90R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7146 as residues: Pro-2 to Thr-10.
HCQCL01R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7147 as residues: Ser-10 to Gly-15.
HCQCL05R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7148 as residues: Thr-24 to Thr-33.
HCQCL14R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7151 as residues: Arg-3 to Gly-13.
HCQCL48R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7159 as residues: Ala-1 to Thr-13.
HCQCL51R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7160 as residues: Pro-9 to Asn-19.
HCQCL55R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7162 as residues: Pro-8 to Asn-18.
HCQCL65R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7165 as residues: Lys-1 to Gly-6, Glu-8 to Arg-13.
HCQCL78R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7169 as residues: Lys-15 to Asn-23.
HCQCL79R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7170 as residues: Pro-1 to Pro-8, Pro-17 to Asp-44.
HCQCO30R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7174 as residues: Ala-17 to Asn-28.
HCQCO53R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7175 as residues: Asn-1 to Gly-11, Gly-16 to Arg-22.
HCQCO66R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7177 as residues: Phe-2 to Asn-11.
HCQCO79R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7178 as residues: Arg-1 to Arg-7.
HCQCP19R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7183 as residues: Arg-8 to Met-13, Leu-16 to Leu-24.
HCQCP30R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7186 as residues: Lys-1 to His-7.
HCQCP89R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7193 as residues: Leu-42 to Ser-47.
HCQCR44R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7198 as residues: Lys-34 to Asn-40.
HCQCT38R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7200 as residues: Arg-18 to Arg-26.
HCQCU08R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7204 as residues: Lys-3 to Trp-8.
HCQCU57R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7208 as residues: Lys-1 to Lys-10.
HCQCU67R	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 7210 as residues: Phe-5 to Leu-13.
HCQCV50 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7215 as residues: Thr-8 to Lys-14, Glu-38 to Thr-50, Arg-56 to Asp-62.
HCQCV91 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7218 as residues: Lys-1 to Phe-11.
HCQCX90 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7225 as residues: Leu-5 to Tyr-11.
HCQDA28 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7228 as residues: Glu-48 to Lys-57.
HCQDA36 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7229 as residues: Met-6 to Ser-14, Ser-24 to Lys-29.
HCQDA66 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7232 as residues: Ala-10 to Thr-15, Arg-20 to Glu-34.
HCQDB17R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7234 as residues: Ala-2 to Gly-15, Cys-20 to Asn-29, Gln-35 to Lys-41, Phe-47 to Lys-59.
HCQDB41R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7237 as residues: Gly-1 to Ala-8.
HCQDB49R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7239 as residues: Phe-8 to Gly-13, Pro-16 to Asn-26, Gln-33 to Thr-38.
HCQDB52R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7240 as residues: Leu-13 to Ser-20.
HCQDB54R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7241 as residues: Pro-5 to Trp-17.
HCQDC12R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7245 as residues: Glu-8 to Asn-13, Arg-16 to Ala-28.
HCQDD35 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7255 as residues: Asn-26 to Tyr-32.
HCQDE68R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7269 as residues: Pro-8 to Asn-18, Leu-27 to Cys-33.
HCQDF44R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7271 as residues: Ser-6 to Val-15.
HCQDF69R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7274 as residues: Ser-19 to Arg-25.
HCQDG40 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7278 as residues: Asn-2 to Val-8, Phe-25 to Leu-30.
HCQDG71 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7280 as residues: Lys-8 to Phe-13.
HCQDG80 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7281 as residues: Ser-4 to Tyr-10.
HCQDH18 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7283 as residues: Asn-31 to Ser-37.
HCQDH60 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7288 as residues: Pro-9 to Asn-19, Gln-26 to Ser-34.
HCQDJ22R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7306 as residues: Gly-9 to Asn-14.
HCQDK50	Preferred epitopes include those comprising a sequence shown in SEQ

R	ID NO. 7320 as residues: Lys-38 to Asp-43.
HCQDK58 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7322 as residues: Lys-1 to Trp-6.
HCQDL36R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7327 as residues: Arg-12 to Ser-20.
HCQDL57R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7331 as residues: Ser-25 to Asp-32.
HCQDL96R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7333 as residues: Ser-8 to Ala-18.
HCQDM58 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7338 as residues: Phe-5 to Ala-10.
HCQDN78 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7342 as residues: Asn-1 to Gly-6, Pro-9 to Ser-14.
HCQDP14R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7351 as residues: Gly-1 to Tyr-13.
HCQDQ80 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7359 as residues: Pro-34 to Ser-40.
HCQDS61R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7372 as residues: Ile-17 to Val-24.
HCQDU60 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7387 as residues: Pro-9 to Asn-19.
HCQDU94 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7390 as residues: Pro-7 to His-19.
HCQDV44 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7393 as residues: Thr-19 to Thr-26, Ala-38 to Arg-43.
HCRMB19 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7419 as residues: His-23 to Gln-29.
HCRMB44 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7420 as residues: Ser-1 to Ser-8.
HCRMB82 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7422 as residues: Pro-1 to Ser-9.
HCRMD33 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7429 as residues: Pro-14 to Asn-21, Pro-23 to Asn-34.
HCRMD57 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7430 as residues: Arg-14 to Ser-30.
HCRMD77 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7431 as residues: Asn-4 to Asn-10.
HCRMF07 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7436 as residues: Arg-1 to Gly-10, Glu-16 to Gln-21.
HCRMF33 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7439 as residues: Pro-3 to Thr-8.
HCRMF93 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7447 as residues: Leu-2 to Arg-9, Glu-23 to His-34.
HCRMG20 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7449 as residues: Ser-15 to His-22.
HCRMI33R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7457 as residues: Phe-4 to Ala-10.
HCRMI60R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7460 as residues: Glu-21 to Gly-41, Ala-75 to Gly-80.

HCRMJ54R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7463 as residues: Pro-13 to Phe-23.
HCRMJ81R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7465 as residues: Phe-15 to Phe-24, Asn-63 to Ala-69, Leu-80 to Pro-85.
HCRMP32 RA	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7472 as residues: Arg-5 to Glu-14, Arg-31 to Gly-36.
HCRMS48 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7477 as residues: Arg-42 to Lys-50.
HCRMT03 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7480 as residues: Phe-5 to Ser-13.
HCRMU21 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7483 as residues: Ser-20 to Glu-28.
HCRMW62 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7497 as residues: Cys-53 to Ser-60.
HCRMY29 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7502 as residues: Arg-1 to Thr-6.
HCRMZ36 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7505 as residues: Pro-7 to Ser-27.
HCRMZ71 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7507 as residues: Gly-1 to Cys-7, Thr-33 to Lys-38.
HCRMZ92 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7508 as residues: Gly-45 to Ile-56.
HCRNB85R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7519 as residues: His-1 to Arg-9.
HCRNC23R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7520 as residues: Lys-3 to Arg-11, Pro-19 to Gly-24, Ser-74 to Trp-79.
HCRNE15R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7527 as residues: Arg-7 to Ser-12.
HCRNE60R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7532 as residues: Glu-1 to Ser-11.
HCRNF01R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7533 as residues: Gly-46 to Thr-52.
HCRNH02R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7538 as residues: Asn-46 to Gly-57.
HCRNI71R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7540 as residues: Lys-1 to Trp-10.
HCRNJ25R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7541 as residues: Asp-10 to His-16, Arg-24 to Trp-29, Lys-40 to Phe-46, Leu-83 to Trp-90, Pro-92 to His-97.
HCRNK40 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7543 as residues: Ile-49 to Asn-55, Ser-69 to His-79.
HCRNK94 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7544 as residues: Met-34 to Pro-48.
HCRNL38R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7546 as residues: Ser-11 to Ser-16, Ala-52 to Glu-60.
HCRNL55R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7548 as residues: Thr-7 to Thr-15.

HCRNM50 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7553 as residues: Ser-18 to Asn-26.
HCRNO49R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7559 as residues: Gly-24 to Arg-36, Pro-57 to Arg-65.
HCRNV70 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7571 as residues: Asn-1 to Lys-6, Ser-14 to Gly-26.
HCRNW29 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7573 as residues: Gly-23 to Ser-28.
HCRNX03 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7577 as residues: Arg-1 to Glu-9.
HCRNZ22R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7581 as residues: Leu-24 to Asp-32.
HCROE81R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7592 as residues: Gly-1 to Thr-8.
HCROE89R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7593 as residues: Gly-13 to His-18.
HCROF67R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7595 as residues: Lys-1 to Asn-19, Thr-61 to Ala-68.
HCROG58R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7599 as residues: Pro-44 to Gly-49.
HCROG62R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7600 as residues: Ser-19 to Pro-26.
HCROH29R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7602 as residues: Thr-34 to Ser-40, Arg-102 to Trp-109.
HCROJ88R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7613 as residues: Arg-26 to Gly-33, Arg-39 to Arg-60.
HCROK42 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7619 as residues: Arg-20 to Met-28.
HCROK47 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7620 as residues: Arg-8 to Pro-13.
HCROM53 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7631 as residues: Val-11 to Gln-17, Pro-41 to Thr-47, Arg-66 to Glu-75.
HCROM56 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7632 as residues: Arg-12 to Asn-17, Cys-26 to Gln-36.
HCRON01R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7636 as residues: Asp-4 to Thr-10.
HCRON04R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7637 as residues: Thr-1 to Pro-9.
HCRON70R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7641 as residues: Gly-1 to Arg-12.
HCROO46R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7643 as residues: Gln-47 to Ser-58.
HCROQ92R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7653 as residues: Ser-16 to Ser-28.
HCROR76R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7656 as residues: Ser-6 to Gly-11.
HCROS08R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7658 as residues: Asn-23 to Asn-29.

HCROT15R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7662 as residues: Pro-26 to Lys-39, Asn-42 to Asn-49.
HCROT84R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7666 as residues: Pro-22 to Gly-28, Gly-37 to Lys-44.
HCROW69R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7674 as residues: Arg-1 to Gly-8, Leu-19 to Pro-25.
HCROX18R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7676 as residues: Gly-1 to Arg-9.
HCROX38R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7678 as residues: Gly-3 to Val-9.
HCROZ45R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7683 as residues: Thr-1 to Gln-9, Thr-19 to Ser-31, Pro-36 to Glu-42, Leu-53 to Ala-63, Asn-92 to Gly-98, Leu-124 to Leu-131.
HCRPA19R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7689 as residues: Phe-62 to His-68.
HCRPA91R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7692 as residues: Gln-15 to Asn-26.
HCRPC30R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7695 as residues: Val-1 to Gly-6.
HCRPC56R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7698 as residues: Arg-1 to Glu-11, Val-27 to Val-35.
HCRPC58R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7699 as residues: Ala-4 to Thr-9.
HCRPE32R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7705 as residues: Asp-1 to Asp-18, Ser-41 to Arg-52.
HCRPE74R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7706 as residues: Met-6 to Gln-17.
HCRPF62R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7708 as residues: Cys-16 to Lys-33.
HCRPG28R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7715 as residues: Pro-26 to Ser-32.
HCRPG37R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7716 as residues: Arg-3 to Arg-9.
HCRPH31R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7719 as residues: Pro-35 to Gly-40.
HCRPH50R A	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7720 as residues: Pro-2 to His-8.
HCRPH58R A	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7721 as residues: Arg-14 to Val-19.
HCRPJ68R A	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7727 as residues: Trp-29 to Asn-42.
HCRPL63R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7733 as residues: Ser-10 to Leu-21, Phe-31 to Lys-36, Ala-54 to Leu-67.
HCRPL79R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7734 as residues: Arg-1 to Leu-6.
HCRPM51R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7737 as residues: Gly-14 to Thr-19, Gly-42 to Trp-48, Asp-63 to Ala-71.

HCRPN29R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7740 as residues: Lys-7 to Cys-12.
HCRPN49R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7742 as residues: Ser-6 to Thr-11, Pro-14 to His-28, Pro-34 to Asp-42, Pro-51 to Thr-60.
HCRPN73R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7743 as residues: Asn-16 to Ala-21.
HCRPO31R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7746 as residues: Gly-25 to Arg-30.
HCRPQ72R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7754 as residues: Pro-12 to Ser-17, Trp-30 to Ala-35, Gln-49 to Gln-55.
HCRPR62R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7757 as residues: Cys-14 to His-20.
HCRPR70R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7758 as residues: Arg-16 to His-24.
HCRPR91R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7759 as residues: Tyr-1 to Ile-6, Gln-16 to Asp-24.
HCRPT82R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7767 as residues: Lys-1 to Lys-7.
HCRPU09R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7769 as residues: Phe-20 to Thr-25.
HCRPV91R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7775 as residues: Glu-19 to Ala-31, Glu-52 to Thr-82, Leu-104 to Gln-110, Arg-125 to Arg-130.
HCRPX71R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7779 as residues: Pro-5 to Ala-11.
HCRPY01R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7780 as residues: Glu-1 to Gly-10, Ala-23 to Phe-33, Gln-59 to Ser-64.
HCRPY91R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7782 as residues: His-9 to Thr-17, Thr-25 to His-31.
HCRQB75R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7785 as residues: Arg-11 to Gly-23.
HCRQC36R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7786 as residues: Arg-53 to Arg-60.
HCRQD29R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7788 as residues: Pro-7 to Ala-15, Ser-32 to Lys-40.
HCRQD47R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7790 as residues: Ser-57 to Arg-64, Glu-71 to Gly-84, Arg-95 to Trp-100.
HCRQJ26R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7804 as residues: Asn-1 to Gly-9.
HCRQL13R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7808 as residues: Glu-22 to Gly-27.
HCRQL65R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7809 as residues: Arg-6 to Thr-11.
HCRQM37R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7810 as residues: Ala-42 to Pro-47, Pro-59 to Ser-66, Leu-79 to

	Arg-84, Gly-114 to Thr-119, Pro-132 to Gly-139.
HCRQM58 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7812 as residues: Glu-1 to Thr-7, Leu-12 to Asn-18.
HCRQM59 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7813 as residues: Glu-6 to Gly-13, Pro-64 to Ala-70.
HCYBA36R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7818 as residues: Tyr-40 to Ser-48.
HCYBD19R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7820 as residues: Ala-18 to Glu-26, Lys-39 to Glu-44, Phe-50 to Ser-55.
HCYBE34R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7822 as residues: Glu-27 to Pro-34, Ser-49 to Gln-54, Ser-56 to Thr-62, Asp-102 to Lys-107, Gly-113 to Glu-119.
HCYBH89R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7826 as residues: Pro-33 to Pro-47.
HCYBH93R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7827 as residues: Ser-11 to Thr-19, Arg-59 to Asp-65.
HDPPE11R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7830 as residues: Pro-1 to Ala-14, Pro-44 to Gly-51.
HDTDS96R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7835 as residues: Ser-17 to Pro-22.
HE8AE77R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7840 as residues: Ile-3 to Asn-9.
HEONL43R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7842 as residues: Arg-1 to Val-10.
HFKHA60R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7845 as residues: Pro-13 to Arg-18, Phe-27 to Glu-37, Ala-45 to Leu-53, Gln-61 to Glu-69, Ser-75 to Ser-82, Gln-84 to Gly-94, Ala-96 to Pro-112.
HFRBW76 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7847 as residues: Thr-2 to Gly-13.
HGBBA17R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7849 as residues: Asp-16 to Asn-22.
HHEQA63R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7853 as residues: Thr-13 to Ser-19, Ile-52 to Thr-59.
HHEWA82 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7854 as residues: Cys-10 to Glu-15.
HHMMA39 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7856 as residues: Arg-15 to Pro-21.
HHMMB13 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7861 as residues: Glu-35 to Val-42.
HHMME20 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7870 as residues: Thr-11 to Ala-17.
HJMBH59R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7881 as residues: Ser-8 to Phe-24.
HKCSB18R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7885 as residues: Arg-12 to Lys-19.
HKCSF11R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7889 as residues: Pro-18 to Ser-26.

HKCSJ63R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7892 as residues: Pro-6 to Gly-12.
HKCTB80R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7898 as residues: Ser-7 to Val-13, Arg-54 to Pro-62.
HKCTD27R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7900 as residues: Thr-9 to Gly-16.
HKLRA55R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7904 as residues: Arg-41 to Arg-47.
HKLSB04R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7912 as residues: Ser-27 to Leu-36, Glu-45 to Gly-52.
HKLSB05R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7913 as residues: Asn-1 to Phe-7, Val-15 to Met-20.
HKLSB41R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7914 as residues: Phe-13 to Ala-27, Gly-70 to Glu-77.
HKLSB76R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7915 as residues: Phe-1 to Gln-10.
HKLSC29R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7917 as residues: Ala-4 to Ser-12.
HKLSD79R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7922 as residues: Ser-8 to Gly-15.
HKLSD93R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7923 as residues: Gly-11 to Gly-17.
HNBTH48R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7928 as residues: Thr-7 to Ser-13.
HNTCO26R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7931 as residues: Arg-1 to Lys-10, Asn-18 to Thr-28.
HOCTA23R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7933 as residues: Phe-17 to Gly-22, Thr-40 to Val-47, Pro-58 to Gly-72, Pro-92 to Trp-109.
HOCTB19R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7936 as residues: Gln-13 to Ser-34.
HOCTB32R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7937 as residues: Arg-1 to Lys-8, Phe-30 to Lys-35.
HOCTC38R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7941 as residues: Ser-6 to Ser-14, Val-16 to Gln-23, Gly-39 to Ser-45, Thr-52 to Ser-58.
HOCTD35R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7946 as residues: Cys-2 to Val-7.
HOCTE12R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7950 as residues: Asn-1 to Val-6, Pro-22 to Phe-29.
HOCTF43R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7953 as residues: Asp-22 to Gly-27, Arg-35 to Pro-43, Asp-63 to Ser-68.
HOHAS78R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7959 as residues: Ala-1 to Cys-20, Arg-29 to Ser-37, Leu-48 to Phe-54.
HOSNW54R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7961 as residues: Pro-1 to Asp-8, Asn-28 to Ser-33.
HPCRD42R	Preferred epitopes include those comprising a sequence shown in SEQ

	ID NO. 7963 as residues: Arg-1 to Glu-6, Arg-52 to Arg-57.
HPFCN76R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7965 as residues: Ser-1 to Cys-16, Pro-30 to Asp-40.
HPJBZ88R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7966 as residues: Pro-17 to Gly-27, Gly-30 to His-36, Phe-44 to Gly-54, Pro-56 to Ala-61.
HSIFC66R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7968 as residues: Glu-8 to Asn-13, Arg-16 to Thr-29.
HSOBF88R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7969 as residues: Asp-1 to Tyr-8.
HSODE15R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7970 as residues: Leu-8 to Ser-15, Gly-21 to Ser-27.
HTXRF56R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7974 as residues: Glu-1 to Arg-6, Ala-14 to Gly-27, Arg-31 to His-37.
HTYND19 RA	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7975 as residues: Glu-1 to Thr-15, Val-21 to Leu-27, Ser-37 to Arg-58, Met-82 to Asn-91.
HWLMA60 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7981 as residues: Leu-10 to Arg-16.
HWLMB42 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7984 as residues: Arg-24 to Arg-41.
HWLMC65 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7985 as residues: Phe-18 to Trp-23.
HWLMC79 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7986 as residues: Thr-30 to Thr-39.
HWLME59 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7989 as residues: Asp-26 to Cys-32.
HWLME69 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7990 as residues: Arg-11 to Gly-17.
HWLME71 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7991 as residues: Gln-1 to Gly-6.
HWLMG12 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7994 as residues: Asn-1 to Gly-10.
HWLMG15 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 7995 as residues: Pro-10 to Thr-16, Arg-39 to Gly-44.
HWLMG57 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8000 as residues: Ser-7 to Gly-17, Asn-35 to His-46.
HWLMG84 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8002 as residues: Ser-3 to Ala-23, Pro-25 to Gly-31, Ala-59 to Gly-80, Pro-83 to His-91, Gly-99 to Gly-110, Pro-112 to Trp-123.
HWLMH50 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8006 as residues: Ile-2 to Gln-7, Glu-21 to Gly-27.
HWLMJ80 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8010 as residues: Leu-65 to Thr-80.
HWLMK20 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8011 as residues: Ser-8 to Pro-19.
HWLMK25 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8012 as residues: Lys-1 to Ser-6.

HWLMK31 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8013 as residues: Arg-1 to Trp-10, Arg-15 to Gly-24.
HWLMK62 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8014 as residues: Gly-1 to Ala-10, Pro-42 to Pro-53.
HWLMM68 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8015 as residues: His-10 to Asn-16.
HWLMQ01 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8023 as residues: Asn-3 to Lys-12.
HWLMR23 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8025 as residues: Gly-9 to Lys-17.
HWLMR69 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8026 as residues: Asp-6 to Glu-13, Leu-63 to Gln-70.
HWLMS31 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8027 as residues: Pro-2 to Leu-7.
HWLMT64 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8030 as residues: Asp-1 to Gln-6.
HWLMU26 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8033 as residues: Pro-21 to Val-26, Val-28 to Val-37, Ser-44 to Tyr-49, Phe-53 to Leu-65.
HWLMV60 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8036 as residues: Ser-27 to Glu-39, Leu-43 to Gln-48.
HWLNH76 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8050 as residues: Cys-8 to His-24, Ser-36 to Arg-44.
HWLNL41 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8055 as residues: Pro-1 to Glu-22, Ala-31 to Asp-39, Glu-65 to Pro-72.
HWLNP65 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8061 as residues: Val-12 to Trp-17, Ile-22 to Ser-28.
HWLNR26 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8063 as residues: Glu-10 to Gly-28.
HWLNY40 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8080 as residues: Pro-1 to Arg-18.
HWLOA09 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8083 as residues: Tyr-13 to Phe-18, Gln-22 to Tyr-27, Pro-74 to Met-81.
HWLOC65 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8087 as residues: Arg-41 to Asn-50.
HWLOF46 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8090 as residues: Arg-11 to Val-19, Thr-28 to Ala-39.
HWLOI17R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8097 as residues: His-21 to Gly-29.
HWLOJ19R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8102 as residues: Ser-20 to Leu-37.
HWLOK12 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8105 as residues: Arg-24 to Asn-29.
HWLOK45 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8107 as residues: His-20 to Pro-26.
HWLON66 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8112 as residues: Phe-1 to Gln-11.

HWLON71 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8113 as residues: Ala-1 to Tyr-8.
HWLOQ52 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8115 as residues: Cys-2 to Asn-8.
HWLOR15 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8117 as residues: Asp-1 to Gly-10, Thr-53 to Asp-59.
HWLOR65 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8118 as residues: Gly-16 to Gln-26, Gly-31 to Lys-37.
HWLOX29 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8132 as residues: Ser-16 to Ser-22.
HWLOY73 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8135 as residues: Pro-1 to Val-11, Pro-13 to Gln-20, Pro-39 to Pro-46, Gln-51 to Ala-73.
HWLOZ87 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8137 as residues: Gln-20 to Ser-27, Gln-42 to Ser-48.
HWLQA28 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8140 as residues: Lys-40 to Asn-55.
HWLQD30 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8147 as residues: Pro-6 to Pro-13, Gly-19 to Lys-39.
HWLQD40 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8148 as residues: Pro-14 to Asn-19, Glu-51 to Asn-57, Ser-67 to Pro-75.
HWLQD46 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8151 as residues: Gly-28 to Leu-33.
HWLQD89 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8152 as residues: Lys-2 to Lys-7.
HWLQH32 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8164 as residues: Asn-19 to Thr-27.
HWLQH58 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8165 as residues: Pro-45 to Asp-52.
HWLQM69 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8169 as residues: Glu-6 to Pro-12.
HWLQP18 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8175 as residues: Ser-2 to Ala-11.
HWLQQ83 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8177 as residues: Ser-26 to Gly-37, Pro-44 to Ser-50.
HWLQR90 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8178 as residues: Gln-1 to Trp-9, Val-17 to Glu-22.
HWLQT52 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8182 as residues: Gly-1 to Ser-10, Arg-16 to Met-22, Ser-24 to Trp-29, Gly-37 to Arg-44, Gly-52 to Ser-59, Arg-67 to Ser-85, Thr-107 to Gly-114.
HWLQU50 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8185 as residues: Tyr-26 to Cys-34, Thr-45 to Asn-50.
HWLRB15 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8191 as residues: Leu-8 to His-14, Ser-17 to Trp-31, Thr-44 to Gln-50, Ala-53 to Ala-61, Thr-72 to Ala-90, Val-116 to Leu-123.
HWLRE01 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8194 as residues: Ser-9 to Asn-19, Asn-34 to Cys-41.

HWLRO35 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8206 as residues: Ile-20 to Thr-29, Lys-39 to Ala-46.
HWLRV63 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8218 as residues: Glu-15 to Cys-26, Arg-34 to Ile-58.
HWLUG53 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8222 as residues: Asn-17 to Lys-27.
HWLUH72 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8225 as residues: Asp-58 to Cys-72, Gln-81 to Glu-89.
HWLUJ19R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8231 as residues: Ser-49 to Ser-55.
HWLUL47 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8236 as residues: Lys-18 to Lys-24.
HWLUL65 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8237 as residues: Asp-1 to His-8.
HWLUQ87 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8254 as residues: Cys-34 to Arg-41.
HWLUR41 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8256 as residues: Ser-24 to Trp-30.
HWLUU88 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8261 as residues: Pro-9 to Gly-20.
HWLUV67 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8264 as residues: Pro-5 to Arg-13.
HWLUZ07 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8269 as residues: Glu-1 to Gly-8.
HWLVD26 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8275 as residues: Arg-11 to Asp-16.
HWLVD74 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8278 as residues: His-1 to Thr-10.
HWLVE21 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8279 as residues: Leu-33 to Glu-40, Lys-52 to Lys-62.
HWLVF34 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8282 as residues: Arg-54 to His-62.
HWLVJ15R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8289 as residues: Phe-38 to Phe-44.
HWLVJ84R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8290 as residues: Asn-2 to Gly-33, Ser-35 to Phe-63.
HWLVK62 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8292 as residues: Ser-1 to Glu-13.
HWLVL10 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8295 as residues: Arg-1 to Thr-8.
HWLVM05 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8298 as residues: Ala-8 to Asn-15.
HWLVN12 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8301 as residues: Gln-1 to Tyr-6.
HWLVV06 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8310 as residues: Arg-1 to Arg-14.
HWLVW89 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8317 as residues: Asn-6 to Gly-11.
HWLVY14	Preferred epitopes include those comprising a sequence shown in SEQ

R	ID NO. 8320 as residues: Ser-1 to Trp-7.
HWLWA14 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8324 as residues: Thr-1 to Trp-9.
HWLWA82 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8325 as residues: Val-1 to Ser-8, Arg-52 to Gly-58.
HWLWB71 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8331 as residues: Cys-28 to Trp-42.
HWLWB77 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8333 as residues: Cys-40 to Trp-47.
HWLWD32 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8334 as residues: Gly-13 to Ala-21.
HWLWD60 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8336 as residues: Tyr-16 to Phe-22.
HWLWE80 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8339 as residues: Gly-1 to Trp-6.
HWLWJ36 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8346 as residues: Asp-11 to Asn-25.
HWLWO57 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8353 as residues: Ser-1 to Phe-6.
HWLWP08 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8357 as residues: Arg-4 to Val-12.
HWLWS28 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8369 as residues: Arg-4 to Tyr-9.
HWLWU27 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8373 as residues: Ala-16 to Phe-21.
HWLWW4 6R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8374 as residues: Ser-6 to Ser-16.
HWLXA13 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8380 as residues: Asp-8 to Ser-17.
HWLXA23 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8381 as residues: Pro-10 to Ile-20.
HWLXJ59R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8388 as residues: Pro-7 to Ser-13.
HWLXN33 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8391 as residues: Glu-1 to Gly-7.
HWLXP33 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8395 as residues: Thr-3 to Lys-13.
HWLXP45 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8396 as residues: Gly-10 to Gly-22, Pro-27 to Arg-35.
HWLXR49 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8403 as residues: Gly-10 to Pro-15.
HWLXT31 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8405 as residues: Gly-10 to Glu-15, Ser-31 to Lys-36.
HWMB46 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8410 as residues: Phe-11 to Lys-17.
HWMBD22 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8411 as residues: Pro-11 to Ala-18.
HWMBD71 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8413 as residues: Asp-4 to Leu-9.

HWMBE36 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8415 as residues: Tyr-12 to Met-18.
HWMBF87 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8416 as residues: Gly-1 to Arg-6.
HWMBG63 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8417 as residues: Glu-1 to Ser-9.
HWMBI08 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8420 as residues: Arg-29 to His-37, Trp-43 to Arg-48.
HWMBK47 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8423 as residues: Asn-6 to His-11, Asn-25 to Cys-30.
HWMBL29 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8425 as residues: Leu-11 to Phe-16.
HWMBL57 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8426 as residues: Glu-46 to Tyr-57.
HWMBL82 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8427 as residues: Leu-27 to Thr-67, His-74 to Asn-79, Ser-83 to Lys-94, Gln-109 to Lys-115, Asp-122 to Tyr-131, Leu-138 to Arg-145, Glu-149 to Lys-154.
HWMBM67 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8430 as residues: Gly-32 to Arg-37, Ala-41 to Asp-47.
HWMBM83 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8431 as residues: Thr-21 to Asn-31.
HWMBN52 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8435 as residues: Thr-21 to Glu-34, Leu-50 to Cys-56.
HWMBP01 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8437 as residues: Ser-9 to Ile-17.
HWMBR40 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8443 as residues: Pro-8 to Glu-19.
HWMBR68 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8446 as residues: Tyr-1 to Trp-6.
HWMBR77 RA	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8448 as residues: Lys-1 to Val-8.
HWMBT23 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8454 as residues: Val-3 to Arg-14.
HWMBV48 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8458 as residues: Pro-51 to Ser-57, Gln-65 to Leu-76.
HWMBW5 4R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8460 as residues: Pro-55 to Glu-63.
HWMBY90 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8466 as residues: Thr-1 to Arg-6.
HWMCB93 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8473 as residues: His-1 to Ala-13.
HWMCCE24 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8480 as residues: Asn-6 to Lys-12.
HWMCF45 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8482 as residues: Pro-10 to Phe-16.
HWMCCH47 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8484 as residues: Pro-16 to Ser-24.
HWMCCH76	Preferred epitopes include those comprising a sequence shown in SEQ

R	ID NO. 8485 as residues: Thr-5 to Val-10.
HWMCI32 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8498 as residues: Lys-5 to Glu-12.
HWMCL55 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8521 as residues: Pro-3 to Asn-8.
HWMCM32 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8528 as residues: Ser-9 to Thr-16.
HWMCM80 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8534 as residues: Pro-2 to Ala-8.
H2CBK69R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8540 as residues: Thr-1 to Ile-6, Gly-35 to Ser-42, Ile-68 to Arg-76.
H2CBD14R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8541 as residues: Asp-57 to Leu-62.
HDTEO77R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8547 as residues: Glu-9 to Gly-17.
HCRNC15R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8548 as residues: Asp-26 to Gln-33, Leu-61 to Cys-66, Thr-143 to Asp-155.
HWLRD05 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8549 as residues: Glu-108 to Asp-119.
HPWBS43R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8550 as residues: Thr-8 to Ala-14.
H2CBU94R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8551 as residues: Gln-7 to Lys-15, Cys-23 to Tyr-31, His-40 to Glu-47, Arg-66 to Cys-79, Lys-91 to Arg-98.
HWMCC56 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 8554 as residues: Pro-30 to Ser-35, Arg-37 to Cys-42, Pro-47 to Gly-53, Arg-61 to Gln-66.

The present application is also directed to proteins containing polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the polypeptide sequence set forth. In preferred embodiments, the application is directed to proteins containing polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to polypeptides having the amino acid sequence of the specific N- and C-terminal deletions. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Preferably, the polynucleotide fragments of the invention encode a polypeptide which demonstrates a functional activity. By a polypeptide demonstrating a "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) protein. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a polypeptide for binding) to an anti-polypeptide antibody], immunogenicity (ability to generate antibody which binds to a cancer specific polypeptide), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide.

The functional activity of the colon and/or colon cancer related polypeptides, and fragments, variants derivatives, and analogs thereof, can be assayed by various methods.

For example, in one embodiment where one is assaying for the ability to bind or compete with full-length polypeptide of the present invention for binding to anti-polypeptide antibody, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

In another embodiment, where a ligand is identified, or the ability of a polypeptide fragment, variant or derivative of the invention to multimerize is being evaluated, binding can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel chromatography, protein affinity chromatography, 5 and affinity blotting. See generally, Phizicky, E., et al., 1995, *Microbiol. Rev.* 59:94-123. In another embodiment, physiological correlates polypeptide of the present invention binding to its substrates (signal transduction) can be assayed.

In addition, assays described herein (see Examples) and otherwise known in the art may routinely be applied to measure the ability of polypeptides of the present invention and 10 fragments, variants derivatives and analogs thereof to elicit polypeptide related biological activity (either in vitro or in vivo). Other methods will be known to the skilled artisan and are within the scope of the invention.

Among the especially preferred fragments of the invention are fragments characterized by structural or functional attributes of polypeptides of the present invention. 15 Such fragments include amino acid residues that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet-forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, surface forming regions, and high antigenic index regions (i.e., 20 containing four or more contiguous amino acids having an antigenic index of greater than or equal to 1.5, as identified using the default parameters of the Jameson-Wolf program) of complete (i.e., full-length) SEQ ID NO:Y. Certain preferred regions include, but are not limited to, regions of the aforementioned types identified by analysis of the amino acid sequence, such preferred regions include; Garnier-Robson predicted alpha-regions, beta- 25 regions, turn-regions, and coil-regions; Chou-Fasman predicted alpha-regions, beta-regions, turn-regions, and coil-regions; Kyte-Doolittle predicted hydrophilic and hydrophobic regions; Eisenberg alpha and beta amphipathic regions; Emini surface-forming regions; and Jameson-Wolf high antigenic index regions, as predicted using the default parameters of these predictive algorithms. Polynucleotides encoding these polypeptides are also encompassed by 30 the invention.

In additional embodiments, the polynucleotides of the invention encode functional attributes of the polypeptides of the present invention. Preferred embodiments of the

invention in this regard include fragments that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions and high antigenic index regions of polypeptides of the present invention. Polypeptide fragments of SEQ ID NO:Y falling within conserved domains are specifically contemplated by the present invention. Moreover, polynucleotide fragments encoding these domains are also contemplated.

Other preferred polypeptide fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

Epitopes & Antibodies

The present invention encompasses colon and/or colon cancer related polypeptides comprising, or alternatively consisting of, an epitope of the polypeptide having an amino acid sequence of SEQ ID NO:Y, or an epitope of the polypeptide sequence encoded by a polynucleotide sequence contained in a clone deposited with the ATCC or encoded by a polynucleotide that hybridizes to the complement of the sequence of SEQ ID NO:Y or contained in a deposited clone under stringent hybridization conditions or lower stringency hybridization conditions as defined supra.

The present invention further encompasses polynucleotide sequences encoding an epitope of a polypeptide sequence of the invention (such as, for example, the sequence disclosed in SEQ ID NO:X) polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to the complementary strand under stringent hybridization conditions or lower stringency hybridization conditions defined supra.

The term "epitopes," as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide encoding this polypeptide. An "immunogenic epitope," as used herein, is defined as a portion of a protein that elicits an

antibody response in an animal, as determined by any method known in the art, for example, by the methods for generating antibodies described *infra*. (See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998- 4002 (1983)). The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody can
5 immunospecifically bind its antigen as determined by any method well known in the art, for example, by the immunoassays described herein. Immunospecific binding excludes non-specific binding but does not necessarily exclude cross- reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic.

Fragments which function as epitopes may be produced by any conventional means.
10 (See, e.g., Houghten, Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985), further described in U.S. Patent No. 4,631,211).

In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at
15 least 50, and, most preferably, between about 15 to about 30 amino acids. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as portions thereof. Antigenic epitopes are useful, for example, to
20 raise antibodies, including monoclonal antibodies, that specifically bind the epitope. Preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these antigenic epitopes. Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., Cell 37:767-778 (1984); Sutcliffe et al., Science 219:660-666 (1983)).

25 Non-limiting examples of antigenic polypeptides or peptides that can be used to generate colon cancer antigen-specific antibodies include a polypeptide comprising the portion(s) of SEQ ID NO:Y specified in Table 8. These polypeptide fragments have been determined to bear antigenic epitopes of the colon and/or colon cancer related proteins of the invention by the analysis of the Jameson-Wolf antigenic index which is included in the
30 DNASTar suite of computer programs. Thus, an antigenic portion of a colon and/or colon cancer related polypeptide of the invention may comprise the portion of SEQ ID NO:Y shown in Table 8 or may comprise the portion shown in Table 8. By "comprise" it is

intended that an antigenic polypeptide may contain the portion of the polypeptide shown in Table 8 but it may contain additional flanking residues on either the amino or carboxyl termini of the recited portion. Such additional flanking sequences are preferably sequences naturally found adjacent to the portion; i.e., contiguous sequence shown in SEQ ID NO:Y.

- 5 Said flanking sequence may, however, be sequences from a heterologous polypeptide, such as from another colon and/or colon cancer related protein described herein or from a heterologous polypeptide not described herein.

Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., supra; Wilson et al., supra; Chow et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle et al., J. Gen. Virol. 66:2347-2354 (1985). Preferred immunogenic epitopes include the immunogenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these immunogenic epitopes. The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

- 20 Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, in vivo immunization, in vitro immunization, and phage display methods. See, e.g., Sutcliffe et al., supra; Wilson et al., supra, and Bittle et al., J. Gen. Virol., 66:2347-2354 (1985). If in vivo immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl- N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier- coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 µg of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an immune
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response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

As one of skill in the art will appreciate, and as discussed above, the polypeptides of the present invention comprising an immunogenic or antigenic epitope can be fused to other polypeptide sequences. For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof), or albumin (including but not limited to recombinant albumin (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)), resulting in chimeric polypeptides. Such fusion proteins may facilitate purification and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., *Nature*, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion disulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., *J. Biochem.*, 270:3958-3964 (1995). Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, *Proc. Natl. Acad. Sci. USA* 88:8972- 897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix

binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto Ni²⁺ nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.

Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of polypeptides corresponding to SEQ ID NO:Y, such methods can be used to generate polypeptides with altered activity, as well as agonists and antagonists of the polypeptides. See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al., Curr. Opin. Biotechnol. 8:724-33 (1997); Harayama, Trends Biotechnol. 16(2):76-82 (1998); Hansson, et al., J. Mol. Biol. 287:265-76 (1999); and Lorenzo and Blasco, Biotechniques 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference in its entirety).

In one embodiment, alteration of polynucleotides corresponding to SEQ ID NO:X and the polypeptides encoded by these polynucleotides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments by homologous or site-specific recombination to generate variation in the polynucleotide sequence. In another embodiment, polynucleotides of the invention, or the encoded polypeptides, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of a polynucleotide encoding a polypeptide of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

25 **Antibodies**

Further polypeptides of the invention relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, polypeptide fragment, or variant of SEQ ID NO:Y, and/or an epitope, of the present invention (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding). Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies

(including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. In preferred embodiments, the immunoglobulin molecules of the invention are IgG1. In other preferred embodiments, the immunoglobulin molecules of the invention are IgG4.

Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')₂, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, sheep rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Patent No. 5,939,598 by Kucherlapati et al.

The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or listed in the Tables and Figures. Preferred epitopes of the invention include those shown in Table 8, as well as polynucleotides that encode these epitopes. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a polypeptide of the present invention are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In specific embodiments, antibodies of the present invention cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific antigenic and/or immunogenic polypeptides disclosed herein. Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or K_d less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

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The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Preferably, antibodies of the present invention bind an antigenic epitope disclosed herein, or a portion thereof. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described supra). In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

The invention also features receptor-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included in the invention are antibodies which activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation, for example, by inducing dimerization of the receptor. The antibodies may be specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities of the peptides of the invention disclosed herein. The above antibody agonists can be made using

methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., *Blood* 92(6):1981-1988 (1998); Chen et al., *Cancer Res.* 58(16):3668-3678 (1998); Harrop et al., *J. Immunol.* 161(4):1786-1794 (1998); Zhu et al., *Cancer Res.* 58(15):3209-3214 (1998); Yoon et al., *J. Immunol.* 160(7):3170-3179 (1998);
5 Prat et al., *J. Cell. Sci.* 111(Pt2):237-247 (1998); Pitard et al., *J. Immunol. Methods* 205(2):177-190 (1997); Liautard et al., *Cytokine* 9(4):233-241 (1997); Carlson et al., *J. Biol. Chem.* 272(17):11295-11301 (1997); Taryman et al., *Neuron* 14(4):755-762 (1995); Muller et al., *Structure* 6(9):1153-1167 (1998); Bartunek et al., *Cytokine* 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

10 Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al., *Antibodies: A Laboratory*
15 *Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically
20 conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

25 The antibodies of the invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by
30 known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation,

metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of- interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art and are discussed in detail in the Examples. In a non-limiting example, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then

assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

Accordingly, the present invention provides methods of generating monoclonal
5 antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

10 Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')₂ fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments). F(ab')₂ fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

15 For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial
20 antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the
25 phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., J. Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995); Kettleborough et al., Eur. J. Immunol. 24:952-958 (1994); Persic et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280 (1994); PCT application No.
30 PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908;

5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')₂ fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., *BioTechniques* 12(6):864-869 (1992); and Sawai et al., *AJRI* 34:26-34 (1995); and Better et al., *Science* 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., *Methods in Enzymology* 203:46-88 (1991); Shu et al., *PNAS* 90:7995-7999 (1993); and Skerra et al., *Science* 240:1038-1040 (1988). For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, *Science* 229:1202 (1985); Oi et al., *BioTechniques* 4:214 (1986); Gillies et al., (1989) *J. Immunol. Methods* 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and a framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Patent No. 5,585,089; Riechmann et al.,

Nature 332:323 (1988), which are incorporated herein by reference in their entirety.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332).

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically

useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, Int. Rev. Immunol. 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., Bio/technology 12:899-903 (1988)).

Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, FASEB J. 7(5):437-444; (1989) and Nissinoff, J. Immunol. 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligands/receptors, and thereby block its biological activity.

Polynucleotides Encoding Antibodies

The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that encode an antibody, preferably, that

specifically binds to a polypeptide of the invention, preferably, an antibody that binds to a polypeptide having the amino acid sequence of SEQ ID NO:Y.

The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide
5 sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., *BioTechniques* 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by
10 PCR.

Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable
15 source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA
20 clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods
25 well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel et al., eds., 1998, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY, which are both incorporated by reference
30 herein in their entirety), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well known in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described supra. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., *J. Mol. Biol.* 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., *Proc. Natl. Acad. Sci.* 81:851-855 (1984); Neuberger et al., *Nature* 312:604-608 (1984); Takeda et al., *Nature* 314:452-454 (1985)) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778; Bird, *Science* 242:423-42 (1988); Huston et al., *Proc. Natl. Acad. Sci. USA* 85:5879-5883 (1988); and Ward et al., *Nature* 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single

chain polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* may also be used (Skerra et al., Science 242:1038- 1041 (1988)).

Methods of Producing Antibodies

5 The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain
10 antibody of the invention), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology
15 using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA
20 techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT
25 Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an
30 antibody of the invention, or a heavy or light chain thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains

may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., *Gene* 45:101 (1986); Cockett et al., *Bio/Technology* 8:2 (1990)).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., *EMBO J.* 2:1791 (1983)), in which the antibody coding sequence may be ligated

individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, Nucleic Acids Res. 13:3101-3109 (1985); Van Heeke & Schuster, J. Biol. Chem. 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

10 In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

15 In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

20 25 30 In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein

products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, 5 eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for 10 example, CRL7030 and Hs578Bst.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control 15 elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their 20 chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

A number of selection systems may be used, including but not limited to the herpes 25 simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk-, hgp^rt- or ap^rt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to 30 methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers

resistance to the aminoglycoside G-418 *Clinical Pharmacy* 12:488-505; Wu and Wu, *Biotherapy* 3:87-95 (1991); Tolstoshev, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596 (1993); Mulligan, *Science* 260:926-932 (1993); and Morgan and Anderson, *Ann. Rev. Biochem.* 62:191-217 (1993); May, 1993, *TIB TECH* 11(5):155-215; and hygromycin, which confers resistance to hygromycin (Santerre et al., *Gene* 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1993); Kriegler, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), *Current Protocols in Human Genetics*, John Wiley & Sons, NY (1994); Colberre-Garapin et al., *J. Mol. Biol.* 150:1 (1981), which are incorporated by reference herein in their entirety.

The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, *The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning*, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., *Mol. Cell. Biol.* 3:257 (1983)).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, *Nature* 322:52 (1986); Kohler, *Proc. Natl. Acad. Sci. USA* 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by

chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies of the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., *supra*, and PCT publication WO 93/21232; EP 439,095; Naramura et al., *Immunol. Lett.* 39:91-99 (1994); U.S. Patent 5,474,981; Gillies et al., *PNAS* 89:1428-1432 (1992); Fell et al., *J. Immunol.* 146:2446-2452(1991), which are incorporated by reference in their entireties.

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851;

5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 91/06570; Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88:10535-10539 (1991); Zheng et al., J. Immunol. 154:5590-5600 (1995); and Vil et al., Proc. Natl. Acad. Sci. USA 89:11337-11341(1992) (said references incorporated by reference in their entireties).

5 As discussed, supra, the polypeptides corresponding to a polypeptide, polypeptide fragment, or a variant of SEQ ID NO:Y may be fused or conjugated to the above antibody portions to increase the in vivo half life of the polypeptides or for use in immunoassays using methods known in the art. Further, the polypeptides corresponding to SEQ ID NO:Y may be fused or conjugated to the above antibody portions to facilitate purification. One reported
10 example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP 394,827; Traunecker et al., Nature 331:84-86 (1988). The polypeptides of the present invention fused or conjugated to an antibody having disulfide- linked dimeric structures (due to the IgG) may also be more efficient in binding
15 and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995)). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP A 232,262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For
20 example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, Bennett et al., J. Molecular Recognition 8:52-58 (1995); Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).

25 Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA
30 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the

"HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)) and the "flag" tag.

The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include ¹²⁵I, ¹³¹I, ¹¹¹In or ⁹⁹Tc.

Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytotoxic agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, ²¹³Bi. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, teniposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g.,

mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis- dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, α -interferon, β -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF- α , TNF- β , AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al.*, *Int. Immunol.*, 6:1567-1574 (1994)), VEGI (See, International Publication No. WO 99/23105), a thrombotic agent or an anti- angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of

Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev. 62:119-58 (1982).

5 Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

 An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a
10 therapeutic.

Immunophenotyping

 The antibodies of the invention may be utilized for immunophenotyping of cell lines and biological samples. The translation product of the gene of the present invention may be
15 useful as a cell specific marker, or more specifically as a cellular marker that is differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies directed against a specific epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the
20 marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Patent 5,985,660; and Morrison *et al.*, *Cell*, 96:737-49 (1999)).

 These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in
25 acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

Assays For Antibody Binding

 The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited

to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, 5 immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

10 Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X- 100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time 15 (e.g., 1-4 hours) at 4° C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to 20 increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

Western blot analysis generally comprises preparing protein samples, electrophoresis 25 of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of 30 interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or

alkaline phosphatase) or radioactive molecule (e.g., ^{32}P or ^{125}I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., ^3H or ^{125}I) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the antigen is incubated with antibody of interest conjugated to a labeled compound (e.g., ^3H or ^{125}I) in the presence of increasing amounts of an unlabeled second antibody.

Therapeutic Uses

The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a polypeptide of the invention, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a polypeptide of the invention includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments

derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides of the invention, including fragments thereof. Preferred binding affinities include those with a dissociation constant or K_d less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, and 10^{-15} M.

Gene Therapy

In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel et al., *Clinical Pharmacy* 12:488-505 (1993); Wu and Wu, *Biotherapy* 3:87-95 (1991); Tolstoshev, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596 (1993); Mulligan, *Science* 260:926-932 (1993); and Morgan and Anderson, *Ann. Rev. Biochem.* 62:191-217 (1993); May, *TIBTECH* 11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1993); and Kriegler, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY (1990).

In a preferred aspect, the compound comprises nucleic acid sequences encoding an antibody, said nucleic acid sequences being part of expression vectors that express the

antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989). In specific embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody.

Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids in vitro, then transplanted into the patient. These two approaches are known, respectively, as in vivo or ex vivo gene therapy.

In a specific embodiment, the nucleic acid sequences are directly administered in vivo, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180; WO 92/22635;

WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989)).

5 In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy
10 are cloned into one or more vectors, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., Biotherapy 6:291-302 (1994), which describes the use of a retroviral vector to deliver the *mdr1* gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., J.
15 Clin. Invest. 93:644-651 (1994); Kiem et al., Blood 83:1467-1473 (1994); Salmons and Gunzberg, Human Gene Therapy 4:129-141 (1993); and Grossman and Wilson, Curr. Opin. in Genetics and Devel. 3:110-114 (1993).

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses
20 naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, Current Opinion in Genetics and Development 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., Human Gene
25 Therapy 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., Science 252:431-434 (1991); Rosenfeld et al., Cell 68:143-155 (1992); Mastrangeli et al., J. Clin. Invest. 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., Gene Therapy 2:775-783 (1995). In a preferred
30 embodiment, adenovirus vectors are used.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., Proc. Soc. Exp. Biol. Med. 204:289-300 (1993); U.S. Patent No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, *Meth. Enzymol.* 217:599-618 (1993); Cohen et al., *Meth. Enzymol.* 217:618-644 (1993); Cline, *Pharmac. Ther.* 29:69-92m (1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as Tlymphocytes, Blymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible

by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication
5 WO 94/08598; Stemple and Anderson, Cell 71:973-985 (1992); Rheinwald, Meth. Cell Bio. 21A:229 (1980); and Pittelkow and Scott, Mayo Clinic Proc. 61:771 (1986)).

In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the
10 appropriate inducer of transcription.

Demonstration of Therapeutic or Prophylactic Activity

The compounds or pharmaceutical compositions of the invention are preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in
15 humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance
20 with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

Therapeutic/Prophylactic Administration and Composition

The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical composition of the invention, preferably an antibody of the invention. In a preferred aspect, the compound is substantially purified (e.g., substantially free from substances that limit its
30 effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

5 Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, 10 intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In 15 addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation 20 with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by 25 means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

In another embodiment, the compound or composition can be delivered in a vesicle, 30 in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler

(eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)

In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*;
5 Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980);
Saudek et al., *N. Engl. J. Med.* 321:574 (1989)). In another embodiment, polymeric
materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise
(eds.), CRC Pres., Boca Raton, Florida (1974); *Controlled Drug Bioavailability, Drug
Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger
10 and Peppas, J., *Macromol. Sci. Rev. Macromol. Chem.* 23:61 (1983); see also Levy et al.,
Science 228:190 (1985); During et al., *Ann. Neurol.* 25:351 (1989); Howard et al.,
J. Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be
placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of
the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, *supra*,
15 vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (*Science*
249:1527-1533 (1990)).

In a specific embodiment where the compound of the invention is a nucleic acid
encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its
20 encoded protein, by constructing it as part of an appropriate nucleic acid expression vector
and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see
U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment
(e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or
transfecting agents, or by administering it in linkage to a homeobox- like peptide which is
25 known to enter the nucleus (see e.g., Joliot et al., *Proc. Natl. Acad. Sci. USA* 88:1864-1868
(1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated
within host cell DNA for expression, by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions
comprise a therapeutically effective amount of a compound, and a pharmaceutically
30 acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means
approved by a regulatory agency of the Federal or a state government or listed in the U.S.
Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more

particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the

composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

Diagnosis and Imaging

Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a polypeptide of interest can be used for diagnostic purposes to detect, diagnose, or monitor diseases, disorders, and/or conditions associated with the aberrant expression and/or activity of a polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant expression.

The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (¹²⁵I, ¹²¹I), carbon (¹⁴C), sulfur (³⁵S), tritium (³H), indium (¹¹²In), and technetium (⁹⁹Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to the polypeptide of interest; b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99m}Tc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

Presence of the labeled molecule can be detected in the patient using methods known in the art for in vivo scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patient using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

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Kits

The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least

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one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or
5 chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of
10 the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or
15 polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

20 In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the
25 solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

The solid surface reagent in the above assay is prepared by known techniques for
30 attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically

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through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

Fusion Proteins

Any colon and/or colon cancer related polypeptide of the invention can be used to generate fusion proteins. For example, a colon and/or colon cancer related polypeptide, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the colon and/or colon cancer related polypeptide can be used to indirectly detect the second protein by binding to the colon and/or colon cancer related polypeptide. Moreover, because secreted proteins target cellular locations based on trafficking signals, the colon and/or colon cancer related polypeptides can be used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to colon and/or colon cancer related polypeptides include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

Moreover, fusion proteins may also be engineered to improve characteristics of the colon and/or colon cancer related polypeptide. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the colon and/or colon cancer related polypeptide to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties may be added to the colon and/or colon cancer related polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the colon and/or colon cancer related protein. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

As one of skill in the art will appreciate, polypeptides of the present invention and the epitope-bearing fragments thereof described above, can be combined with heterologous polypeptide sequences. For example, the polypeptides of the present invention may be fused with heterologous polypeptide sequences, for example, the polypeptides of the present

invention may be fused with parts of the constant domain of immunoglobulins (IgA, IgE, IgG, IgM) or portions thereof (CH1, CH2, CH3, and any combination thereof, including both entire domains and portions thereof), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. One reported example
5 describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP A 394,827; Traunecker et al., *Nature* 331:84-86 (1988).) Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or
10 protein fragment alone. (Fountoulakis et al., *J. Biochem.* 270:3958-3964 (1995).)

Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved
15 pharmacokinetic properties. (EP-A 0232 262.) Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify
20 antagonists of hIL-5. (See, D. Bennett et al., *J. Molecular Recognition* 8:52-58 (1995); K. Johanson et al., *J. Biol. Chem.* 270:9459-9471 (1995).)

Moreover, the colon and/or colon cancer related polypeptides can be fused to marker sequences, such as a peptide which facilitates purification of any colon and/or colon cancer related polypeptide. In preferred embodiments, the marker amino acid sequence is a hexa-
25 histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., *Proc. Natl. Acad. Sci. USA* 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza
30 hemagglutinin protein. (Wilson et al., *Cell* 37:767 (1984).)

Thus, any of these above fusions can be engineered using the colon and/or colon cancer related polynucleotides or the polypeptides.

Vectors, Host Cells, and Protein Production

The present invention also relates to vectors containing the polynucleotide of the present invention, host cells, and the production of polypeptides by recombinant techniques.

5 The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

The colon and/or colon cancer related polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is
10 introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the E. coli lac, trp, phoA and tac promoters, the SV40
15 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon
20 (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in E. coli and other bacteria. Representative examples of appropriate hosts include,
25 but are not limited to, bacterial cells, such as E. coli, Streptomyces and Salmonella typhimurium cells; fungal cells, such as yeast cells (e.g., Saccharomyces cerevisiae or Pichia pastoris (ATCC Accession No. 201178)); insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in
30 the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a,

pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia.

- 5 Preferred expression vectors for use in yeast systems include, but are not limited to pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalph, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlsbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.

- 10 Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (1986). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

- 15 A polypeptide of this invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid
20 chromatography ("HPLC") is employed for purification.

- Polypeptides of the present invention can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast,
25 higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded
30 by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal

process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

In one embodiment, the yeast *Pichia pastoris* is used to express any colon and/or colon cancer related protein of the invention in a eukaryotic system. *Pichia pastoris* is a methylotrophic yeast which can metabolize methanol as its sole carbon source. A main step in the methanol metabolism pathway is the oxidation of methanol to formaldehyde using O₂. This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, *Pichia pastoris* must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for O₂. Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (*AOX1*) is highly active. In the presence of methanol, alcohol oxidase produced from the *AOX1* gene comprises up to approximately 30% of the total soluble protein in *Pichia pastoris*. See, Ellis, S.B., et al., *Mol. Cell. Biol.* 5:1111-21 (1985); Koutz, P.J., et al., *Yeast* 5:167-77 (1989); Tschopp, J.F., et al., *Nucl. Acids Res.* 15:3859-76 (1987). Thus, a heterologous coding sequence, such as, for example, a colon and/or colon cancer related polynucleotide of the present invention, under the transcriptional regulation of all or part of the *AOX1* regulatory sequence is expressed at exceptionally high levels in *Pichia* yeast grown in the presence of methanol.

In one example, the plasmid vector pPIC9K is used to express DNA encoding a colon and/or colon cancer related polypeptide of the invention, as set forth herein, in a *Pichea* yeast system essentially as described in "*Pichia* Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of a colon and/or colon cancer related protein of the invention by virtue of the strong *AOX1* promoter linked to the *Pichia pastoris* alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.

Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG as required.

In another embodiment, high-level expression of a heterologous coding sequence, such as, for example, a colon and/or colon cancer related polynucleotide of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an expression vector such as, for example, pGAPZ or pGAPZalpha, and
5 growing the yeast culture in the absence of methanol.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., coding sequence), and/or to include genetic
10 material (e.g., heterologous polynucleotide sequences) that is operably associated with polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous polynucleotide sequences via homologous recombination (see, e.g., U.S. Patent No.
15 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

In addition, polypeptides of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, *Proteins: Structures and Molecular Principles*, W.H. Freeman & Co., N.Y., and Hunkapiller et al., *Nature*, 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of a polypeptide can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or
25 chemical amino acid analogs can be introduced as a substitution or addition into the polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, α -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, γ -Abu, ϵ -Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline,
30 hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β -alanine, fluoro-amino acids, designer amino acids such as β -methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids,

and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

The invention encompasses polypeptides of the present invention which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH₄; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

Additional post-translational modifications encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

Also provided by the invention are chemically modified derivatives of the polypeptides of the invention which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the

ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 kDa.

As noted above, the polyethylene glycol may have a branched structure. Branched polyethylene glycols are described, for example, in U.S. Patent No. 5,643,575; Morpurgo *et al.*, *Appl. Biochem. Biotechnol.* 56:59-72 (1996); Vorobjev *et al.*, *Nucleosides Nucleotides* 18:2745-2750 (1999); and Caliceti *et al.*, *Bioconjug. Chem.* 10:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik *et al.*, *Exp. Hematol.* 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

As suggested above, polyethylene glycol may be attached to proteins via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to a proteins via covalent bonds to lysine, histidine, aspartic acid, glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine, histidine, aspartic acid, glutamic acid, or

cysteine) of the protein or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the protein.

One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

As indicated above, pegylation of the proteins of the invention may be accomplished by any number of means. For example, polyethylene glycol may be attached to the protein either directly or by an intervening linker. Linkerless systems for attaching polyethylene glycol to proteins are described in Delgado *et al.*, *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992); Francis *et al.*, *Intern. J. of Hematol.* 68:1-18 (1998); U.S. Patent No. 4,002,531; U.S. Patent No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference.

One system for attaching polyethylene glycol directly to amino acid residues of proteins without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchloride ($\text{ClSO}_2\text{CH}_2\text{CF}_3$). Upon reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to amine groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates produced by reacting proteins of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoroethane sulphonyl group.

Polyethylene glycol can also be attached to proteins using a number of different intervening linkers. For example, U.S. Patent No. 5,612,460, the entire disclosure of which is

incorporated herein by reference, discloses urethane linkers for connecting polyethylene glycol to proteins. Protein-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the protein by a linker can also be produced by reaction of proteins with compounds such as MPEG-succinimidylsuccinate, MPEG activated with
5 1,1'-carbonyldiimidazole, MPEG-2,4,5-trichloropenylcarbonate, MPEG-p-nitrophenolcarbonate, and various MPEG-succinate derivatives. A number additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to proteins are described in WO 98/32466, the entire disclosure of which is incorporated herein by reference. Pegylated protein products produced using the reaction chemistries set out
10 herein are included within the scope of the invention.

The number of polyethylene glycol moieties attached to each protein of the invention (*i.e.*, the degree of substitution) may also vary. For example, the pegylated proteins of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution within ranges
15 such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per protein molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado *et al.*, *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992).

The polypeptides of the invention may be in monomers or multimers (*i.e.*, dimers, trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and multimers of the polypeptides of the invention, their preparation, and compositions (preferably, Therapeutics) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional
20 embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.
25

Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only polypeptides corresponding to the amino acid sequence of SEQ ID NO:Y or encoded by the cDNA contained in the deposited clone (including fragments, variants, splice variants, and fusion proteins,
30 corresponding to these as described herein). These homomers may contain polypeptides having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer containing only polypeptides having an identical amino acid

sequence. In another specific embodiment, a homomer of the invention is a multimer containing polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (e.g., containing polypeptides having identical or different amino acid sequences) or a homotrimer (e.g., containing polypeptides having identical and/or different amino acid sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

As used herein, the term heteromer refers to a multimer containing one or more heterologous polypeptides (i.e., polypeptides of different proteins) in addition to the polypeptides of the invention. In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example, homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed when polypeptides of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the polypeptides of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence (e.g., that recited in SEQ ID NO:Y, or contained in the polypeptide encoded by the clone). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a fusion protein. In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925). In a specific example, the

covalent associations are between the heterologous sequence contained in a Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from another protein that is capable of forming covalently associated multimers, such as for example, osteoprotegerin (see, e.g., International Publication NO: WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple polypeptides of the invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

Another method for preparing multimer polypeptides of the invention involves use of polypeptides of the invention fused to a leucine zipper or isoleucine zipper polypeptide sequence. Leucine zipper and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., Science 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric proteins of the invention are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a polypeptide of the invention fused to a polypeptide sequence that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric fusion protein is recovered from the culture supernatant using techniques known in the art.

Trimeric polypeptides of the invention may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties and isoleucine moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (FEBS Letters 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by reference. Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric polypeptides of the invention.

In another example, proteins of the invention are associated by interactions between Flag® polypeptide sequence contained in fusion proteins of the invention containing Flag® polypeptide sequence. In a further embodiment, associations proteins of the invention are associated by interactions between heterologous polypeptide sequence contained in Flag® fusion proteins of the invention and anti-Flag® antibody.

The multimers of the invention may be generated using chemical techniques known in the art. For example, polypeptides desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues located within the sequence of the polypeptides desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely modified by the addition of cysteine or biotin to the C-terminus or N-terminus of the polypeptide and techniques known in the art may be applied to generate multimers containing one or more of these modified polypeptides (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the polypeptide components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, polypeptides contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described herein or

otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain (or hydrophobic or signal peptide) and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

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Uses of the Polynucleotides

Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

10 The colon and/or colon cancer related polynucleotides of the present invention are useful for chromosome identification. There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Each polynucleotide of the present invention can be used as a chromosome marker.

15 Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the sequences shown in SEQ ID NO:X. Primers can be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene
20 corresponding to the SEQ ID NO:X will yield an amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that
25 can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, and preselection by hybridization to construct chromosome specific-cDNA libraries, and computer mapping techniques (See, e.g., Shuler, Trends Biotechnol 16:456-459 (1998) which is hereby incorporated by reference in its entirety).

Precise chromosomal location of the polynucleotides can also be achieved using
30 fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-

4,000 bp are preferred. For a review of this technique, see Verma et al., "Human Chromosomes: a Manual of Basic Techniques," Pergamon Press, New York (1988).

For chromosome mapping, the polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes). Preferred polynucleotides correspond to the noncoding regions of the cDNAs because the coding sequences are more likely conserved within gene families, thus increasing the chance of cross hybridization during chromosomal mapping.

Thus, the present invention also provides a method for chromosomal localization which involves (a) preparing PCR primers from colon and/or colon cancer related polynucleotide sequences in Table 1 and (b) screening somatic cell hybrids containing individual chromosomes.

The polynucleotides of the present invention would likewise be useful for radiation hybrid mapping, HAPPY mapping, and long range restriction mapping. For a review of these techniques and others known in the art, see, e.g., Dear, "Genome Mapping: A Practical Approach," IRL Press at Oxford University Press, London (1997); Aydin, J. Mol. Med. 77:691-694 (1999); Hacia et al., Mol. Psychiatry 3:483-492 (1998); Herrick et al., Chromosome Res. 7:409-423 (1999); Hamilton et al., Methods Cell Biol. 62:265-280 (2000); and/or Ott, J. Hered. 90:68-70 (1999) each of which is hereby incorporated by reference in its entirety.

Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a particular disease. (Disease mapping data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library) .) Assuming 1 megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

Thus, once coinheritance is established, differences in the colon and/or colon cancer related polynucleotide and the corresponding gene between affected and unaffected individuals can be examined. First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in

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some or all affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the polypeptide and the corresponding gene from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide
5 can be used for further linkage analysis.

Thus, the invention provides a method of detecting increased or decreased expression levels of the colon and/or colon cancer related polynucleotides in affected individuals as compared to unaffected individuals using polynucleotides of the present invention and techniques known in the art, including but not limited to the method described in Example
10 11. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

Thus, the invention also provides a diagnostic method useful during diagnosis of a tissue related disorder, including cancers, involving measuring the expression level of colon and/or colon cancer related polynucleotides in colon or colon cancer tissues or other cells or
15 body fluid from an individual and comparing the measured gene expression level with a standard colon and/or colon cancer related polynucleotide expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of a colon related disorder, including colon cancer, or a specific tissue related disorder.

In still another embodiment, the invention includes a kit for analyzing samples for the
20 presence of proliferative and/or cancerous polynucleotides derived from a test subject. In a general embodiment, the kit includes at least one polynucleotide probe containing a nucleotide sequence that will specifically hybridize with a colon and/or colon cancer related polynucleotide and a suitable container. In a specific embodiment, the kit includes two polynucleotide probes defining an internal region of the colon and/or colon cancer related
25 polynucleotide, where each probe has one strand containing a 31'-mer-end internal to the region. In a further embodiment, the probes may be useful as primers for polymerase chain reaction amplification.

Where a diagnosis of a specific tissue related disorder, including, for example, diagnosis of a tumor, has already been made according to conventional methods, the present
30 invention is useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed colon and/or colon cancer related polynucleotide expression will experience a

worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

By "measuring the expression level of colon and/or colon cancer related polynucleotides" is intended qualitatively or quantitatively measuring or estimating the level of the colon and/or colon cancer related polypeptide or the level of the mRNA encoding the colon and/or colon cancer related polypeptide in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the colon and/or colon cancer related polypeptide level or mRNA level in a second biological sample). Preferably, the colon and/or colon cancer related polypeptide level or mRNA level in the first biological sample is measured or estimated and compared to a standard colon and/or colon cancer related polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the specific tissue related disorder or being determined by averaging levels from a population of individuals not having a specific tissue related disorder. As will be appreciated in the art, once a standard colon and/or colon cancer related polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which contains colon and/or colon cancer related polypeptide or mRNA. As indicated, biological samples include body fluids (such as sera, plasma, urine, bile, vaginal pool, semen, lymph, synovial fluid and spinal fluid) which contain the colon and/or colon cancer related polypeptide, and tissue sources found to express the colon and/or colon cancer related polypeptide including colon and/or colon cancer. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

The method(s) provided above may preferably be applied in a diagnostic method and/or kits in which colon and/or colon cancer related polynucleotides and/or polypeptides are attached to a solid support. In one exemplary method, the support may be a "gene chip" or a "biological chip" as described in US Patents 5,837,832, 5,874,219, and 5,856,174. Further, such a gene chip with colon and/or colon cancer related polynucleotides attached may be used to identify polymorphisms between the colon and/or colon cancer related polynucleotide sequences, with polynucleotides isolated from a test subject. The knowledge

of such polymorphisms (i.e. their location, as well as, their existence) would be beneficial in identifying disease loci for many disorders, such as for example, in reproductive disorders, neural disorders, immune system disorders, muscular disorders, gastrointestinal disorders, pulmonary disorders, cardiovascular disorders, renal disorders, proliferative disorders, and/or cancerous diseases and conditions. Such a method is described in US Patents 5,858,659 and 5,856,104. The US Patents referenced supra are hereby incorporated by reference in their entirety herein.

The present invention encompasses colon and/or colon cancer related polynucleotides that are chemically synthesized, or reproduced as peptide nucleic acids (PNA), or according to other methods known in the art. The use of PNAs would serve as the preferred form if the colon and/or colon cancer related polynucleotides are incorporated onto a solid support, or gene chip. For the purposes of the present invention, a peptide nucleic acid (PNA) is a polyamide type of DNA analog and the monomeric units for adenine, guanine, thymine and cytosine are available commercially (Perceptive Biosystems). Certain components of DNA, such as phosphorus, phosphorus oxides, or deoxyribose derivatives, are not present in PNAs. As disclosed by P. E. Nielsen, M. Egholm, R. H. Berg and O. Buchardt, Science 254, 1497 (1991); and M. Egholm, O. Buchardt, L. Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, and P. E. Nielsen, Nature 365, 666 (1993), PNAs bind specifically and tightly to complementary DNA strands and are not degraded by nucleases. In fact, PNA binds more strongly to DNA than DNA itself does. This is probably because there is no electrostatic repulsion between the two strands, and also the polyamide backbone is more flexible. Because of this, PNA/DNA duplexes bind under a wider range of stringency conditions than DNA/DNA duplexes, making it easier to perform multiplex hybridization. Smaller probes can be used than with DNA due to the strong binding. In addition, it is more likely that single base mismatches can be determined with PNA/DNA hybridization because a single mismatch in a PNA/DNA 15-mer lowers the melting point ($T_{sub.m}$) by 8°-20° C, vs. 4°-16° C for the DNA/DNA 15-mer duplex. Also, the absence of charge groups in PNA means that hybridization can be done at low ionic strengths and reduce possible interference by salt during the analysis.

The present invention have uses which include, but are not limited to, detecting cancer in mammals. In particular the invention is useful during diagnosis of pathological cell proliferative neoplasias which include, but are not limited to: acute myelogenous leukemias

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including acute monocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute erythroleukemia, acute megakaryocytic leukemia, and acute undifferentiated leukemia, etc.; and chronic myelogenous leukemias including chronic myelomonocytic leukemia, chronic granulocytic leukemia, etc. Preferred mammals include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits and humans. Particularly preferred are humans.

Pathological cell proliferative disorders are often associated with inappropriate activation of proto-oncogenes. (Germann, E. P. et al., "The Etiology of Acute Leukemia: Molecular Genetics and Viral Oncology," in *Neoplastic Diseases of the Blood*, Vol 1., Wiernik, P. H. et al. eds., 161-182 (1985)). Neoplasias are now believed to result from the qualitative alteration of a normal cellular gene product, or from the quantitative modification of gene expression by insertion into the chromosome of a viral sequence, by chromosomal translocation of a gene to a more actively transcribed region, or by some other mechanism. (Germann et al., supra) It is likely that mutated or altered expression of specific genes is involved in the pathogenesis of some leukemias, among other tissues and cell types. (Germann et al., supra) Indeed, the human counterparts of the oncogenes involved in some animal neoplasias have been amplified or translocated in some cases of human leukemia and carcinoma. (Germann et al., supra)

For example, c-myc expression is highly amplified in the non-lymphocytic leukemia cell line HL-60. When HL-60 cells are chemically induced to stop proliferation, the level of c-myc is found to be downregulated. (International Publication Number WO 91/15580) However, it has been shown that exposure of HL-60 cells to a DNA construct that is complementary to the 5' end of c-myc or c-myb blocks translation of the corresponding mRNAs which downregulates expression of the c-myc or c-myb proteins and causes arrest of cell proliferation and differentiation of the treated cells. (International Publication Number WO 91/15580; Wickstrom et al., *Proc. Natl. Acad. Sci.* 85:1028 (1988); Anfossi et al., *Proc. Natl. Acad. Sci.* 86:3379 (1989)). However, the skilled artisan would appreciate the present invention's usefulness would not be limited to treatment of proliferative disorders of hematopoietic cells and tissues, in light of the numerous cells and cell types of varying origins which are known to exhibit proliferative phenotypes.

In addition to the foregoing, a colon and/or colon cancer related polynucleotide can be used to control gene expression through triple helix formation or through antisense DNA or

RNA. Antisense techniques are discussed, for example, in Okano, J. *Neurochem.* 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al., *Nucleic Acids Research* 6: 3073 (1979); Cooney et al., *Science* 241: 456 (1988); and Dervan et al., *Science* 251: 1360 (1991). Both methods rely on binding of the polynucleotide to a complementary DNA or RNA. For these techniques, preferred polynucleotides are usually oligonucleotides 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix - see Lee et al., *Nucl. Acids Res.* 6:3073 (1979); Cooney et al., *Science* 241:456 (1988); and Dervan et al., *Science* 251:1360 (1991)) or to the mRNA itself (antisense - Okano, J. *Neurochem.* 56:560 (1991); Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988).) Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. The oligonucleotide described above can also be delivered to cells such that the antisense RNA or DNA may be expressed in vivo to inhibit production of colon and/or colon cancer related antigens. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat disease, and in particular, for the treatment of proliferative diseases and/or conditions.

Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the present invention offer a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell.

The polynucleotides are also useful for identifying individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The polynucleotides of the present invention can be used as additional DNA markers for RFLP.

The polynucleotides of the present invention can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique,
5 individuals can be identified because each individual will have a unique set of DNA sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

Forensic biology also benefits from using DNA-based identification techniques as
10 disclosed herein. DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, synovial fluid, amniotic fluid, breast milk, lymph, pulmonary sputum or surfactant, urine, fecal matter, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQa class II HLA gene, are used in forensic biology to identify
15 individuals. (Erlich, H., PCR Technology, Freeman and Co. (1992).) Once these specific polymorphic loci are amplified, they are digested with one or more restriction enzymes, yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene. Similarly, polynucleotides of the present invention can be used as polymorphic markers for forensic purposes.

20 There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to tissues, including but not limited to those shown in Table 3 prepared from the sequences of the present invention. Panels of such reagents can identify tissue by species and/or by organ
25 type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

The polynucleotides of the present invention are also useful as hybridization probes for differential identification of the tissue(s) or cell type(s) present in a biological sample. Similarly, polypeptides and antibodies directed to polypeptides of the present invention are
30 useful to provide immunological probes for differential identification of the tissue(s) (e.g., immunohistochemistry assays) or cell type(s) (e.g., immunocytochemistry assays). In addition, for a number of disorders of the above tissues or cells, significantly higher or lower

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levels of gene expression of the polynucleotides/polypeptides of the present invention may be detected in certain tissues (e.g., tissues expressing polypeptides and/or polynucleotides of the present invention and/or cancerous and/or wounded tissues) or bodily fluids (e.g., vaginal pool, lymph, serum, plasma, urine, synovial fluid or spinal fluid) taken
5 from an individual having such a disorder, relative to a "standard" gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

Thus, the invention provides a diagnostic method of a disorder, which involves: (a) assaying gene expression level in cells or body fluid of an individual; (b) comparing the gene expression level with a standard gene expression level, whereby an increase or decrease in
10 the assayed gene expression level compared to the standard expression level is indicative of disorder.

In the very least, the polynucleotides of the present invention can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the
15 process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

Uses of the Polypeptides

20 Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

Polypeptides and antibodies directed to polypeptides of the present invention are useful to provide immunological probes for differential identification of the tissue(s) (e.g., immunohistochemistry assays such as, for example, ABC immunoperoxidase (Hsu et al., J.
25 Histochem. Cytochem. 29:577-580 (1981)) or cell type(s) (e.g., immunocytochemistry assays).

Antibodies can be used to assay levels of polypeptides encoded by polynucleotides of the invention in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen,
30 et al., J. Cell. Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay

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labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (^{131}I , ^{125}I , ^{123}I , ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium ($^{115\text{m}}\text{In}$, $^{113\text{m}}\text{In}$, ^{112}In , ^{111}In), and technetium (^{99}Tc , $^{99\text{m}}\text{Tc}$), thallium (^{201}Tl), gallium (^{68}Ga , ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm ,
5 ^{177}Lu , ^{159}Gd , ^{149}Pm , ^{140}La , ^{175}Yb , ^{166}Ho , ^{90}Y , ^{47}Sc , ^{186}Re , ^{188}Re , ^{142}Pr , ^{105}Rh , ^{97}Ru ; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying colon and/or colon cancer related polypeptide levels in a biological sample, proteins can also be detected in vivo by imaging. Antibody labels or
10 markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant
15 hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ^{131}I , ^{112}In , $^{99\text{m}}\text{Tc}$, (^{131}I , ^{125}I , ^{123}I , ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium ($^{115\text{m}}\text{In}$, $^{113\text{m}}\text{In}$, ^{112}In , ^{111}In), and technetium (^{99}Tc , $^{99\text{m}}\text{Tc}$), thallium (^{201}Tl), gallium (^{68}Ga , ^{67}Ga), palladium (^{103}Pd),
20 molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F , ^{153}Sm , ^{177}Lu , ^{159}Gd , ^{149}Pm , ^{140}La , ^{175}Yb , ^{166}Ho , ^{90}Y , ^{47}Sc , ^{186}Re , ^{188}Re , ^{142}Pr , ^{105}Rh , ^{97}Ru), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for a digestive system disorder, including but not limited to disorders or diseases of the colon such as colon cancer.
25 It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of $^{99\text{m}}\text{Tc}$. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which express the
30 polypeptide encoded by a polynucleotide of the invention. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and

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Their Fragments" (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (e.g.,
5 polypeptides encoded by colon and/or colon cancer related polynucleotides of the invention and/or antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g.,
10 DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention in association with toxins or cytotoxic prodrugs.

15 By "toxin" is meant one or more compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art,
20 compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNase, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. "Toxin" also includes a cytostatic or cytocidal agent, a therapeutic agent or a
25 radioactive metal ion, e.g., alpha-emitters such as, for example, ^{213}Bi , or other radioisotopes such as, for example, ^{103}Pd , ^{133}Xe , ^{131}I , ^{68}Ge , ^{57}Co , ^{65}Zn , ^{85}Sr , ^{32}P , ^{35}S , ^{90}Y , ^{153}Sm , ^{153}Gd , ^{160}Yb , ^{51}Cr , ^{54}Mn , ^{75}Se , ^{113}Sn , $^{90}\text{Yttrium}$, ^{117}Tin , $^{186}\text{Rhenium}$, $^{166}\text{Holmium}$, and $^{188}\text{Rhenium}$; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

30 Techniques known in the art may be applied to label polypeptides of the invention (including antibodies). Such techniques include, but are not limited to, the use of bifunctional conjugating agents (see e.g., U.S. Patent Nos. 5,756,065; 5,714,631; 5,696,239;

5,652,361; 5,505,931; 5,489,425; 5,435,990; 5,428,139; 5,342,604; 5,274,119; 4,994,560; and 5,808,003; the contents of each of which are hereby incorporated by reference in its entirety).

Thus, the invention provides a diagnostic method of a disorder, which involves (a)
5 assaying the expression level of a colon and/or colon cancer related polypeptide of the present invention in cells or body fluid of an individual, or more preferably, assaying the expression level of a colon and/or colon cancer related polypeptide of the present invention in colon and/or colon cancer tissues or associated bodily fluid of an individual; and (b)
10 comparing the assayed polypeptide expression level with a standard polypeptide expression level, whereby an increase or decrease in the assayed polypeptide expression level compared to the standard expression level is indicative of a disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive
15 diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Moreover, colon and/or colon cancer related polypeptides of the present invention can be used to treat or prevent diseases or conditions such as, for example, gastrointestinal
20 disorders, reproductive disorders, neural disorders, immune system disorders, muscular disorders, pulmonary disorders, cardiovascular disorders, renal disorders, proliferative disorders, and/or cancerous diseases and conditions. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of
25 a different polypeptide (e.g., hemoglobin S for hemoglobin B, SOD, catalase, DNA repair proteins), to inhibit the activity of a polypeptide (e.g., an oncogene or tumor suppressor), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel
30 growth inhibition, enhancement of the immune response to proliferative cells or tissues).

Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat disease (as described supra, and elsewhere herein). For example, administration

of an antibody directed to a polypeptide of the present invention can bind, and/or neutralize the polypeptide, and/or reduce overproduction of the polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

5 At the very least, the polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Polypeptides can also be used to raise antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, the polypeptides of the present
10 invention can be used to test the following biological activities.

Gene Therapy Methods

 Another aspect of the present invention is to gene therapy methods for treating disorders, diseases and conditions. The gene therapy methods relate to the introduction of
15 nucleic acid (DNA, RNA and antisense DNA or RNA) sequences into an animal to achieve expression of the polypeptide of the present invention. This method requires a polynucleotide which codes for a polypeptide operatively linked to a promoter and any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques are known in the art, see, for example, WO90/11092, which
20 is herein incorporated by reference.

 Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) comprising a promoter operably linked to a polynucleotide ex vivo, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, see Belldgrun, A., et al., J. Natl. Cancer
25 Inst. 85: 207-216 (1993); Ferrantini, M. et al., Cancer Research 53: 1107-1112 (1993); Ferrantini, M. et al., J. Immunology 153: 4604-4615 (1994); Kaido, T., et al., Int. J. Cancer 60: 221-229 (1995); Ogura, H., et al., Cancer Research 50: 5102-5106 (1990); Santodonato, L., et al., Human Gene Therapy 7:1-10 (1996); Santodonato, L., et al., Gene Therapy 4:1246-1255 (1997); and Zhang, J.-F. et al., Cancer Gene Therapy 3: 31-38 (1996)), which are herein
30 incorporated by reference. In one embodiment, the cells which are engineered are arterial cells. The arterial cells may be reintroduced into the patient through direct injection to the artery, the tissues surrounding the artery, or through catheter injection.

As discussed in more detail below, the polynucleotide constructs can be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, and the like). The polynucleotide constructs may be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

In one embodiment, the polynucleotide is delivered as a naked polynucleotide. The term "naked" polynucleotide, DNA or RNA refers to sequences that are free from any delivery vehicle that acts to assist, promote or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotides can also be delivered in liposome formulations and lipofectin formulations and the like can be prepared by methods well known to those skilled in the art. Such methods are described, for example, in U.S. Patent Nos. 5,593,972, 5,589,466, and 5,580,859, which are herein incorporated by reference.

The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Appropriate vectors include pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; pSVK3, pBPV, pMSG and pSVL available from Pharmacia; and pEF1/V5, pcDNA3.1, and pRc/CMV2 available from Invitrogen. Other suitable vectors will be readily apparent to the skilled artisan.

Any strong promoter known to those skilled in the art can be used for driving the expression of polynucleotide sequence. Suitable promoters include adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; the β -actin promoter; and human growth hormone promoters. The promoter also may be the native promoter for the polypeptide of the present invention.

Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular, fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. In vivo muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked nucleic acid sequence injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 mg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration.

The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked DNA constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The naked polynucleotides are delivered by any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, and so-called "gene guns". These delivery methods are known in the art.

The constructs may also be delivered with delivery vehicles such as viral sequences, viral particles, liposome formulations, lipofectin, precipitating agents, etc. Such methods of delivery are known in the art.

In certain embodiments, the polynucleotide constructs are complexed in a liposome preparation. Liposomal preparations for use in the instant invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. However, cationic liposomes are particularly preferred because a tight charge complex can be formed between the cationic liposome and the polyanionic nucleic acid. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner et al., Proc. Natl. Acad. Sci. USA (1987) 84:7413-7416, which is herein incorporated by reference); mRNA (Malone et al., Proc. Natl. Acad. Sci. USA (1989) 86:6077-6081, which is herein incorporated by reference); and purified transcription factors (Debs et al., J. Biol. Chem. (1990) 265:10189-10192, which is herein incorporated by reference), in functional form.

Cationic liposomes are readily available. For example, N[1-2,3-dioleoyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are particularly useful and are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, N.Y. (See, also, Felgner et al., Proc. Natl. Acad. Sci. USA (1987) 84:7413-7416, which is herein incorporated by reference). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer).

Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. PCT Publication No. WO 90/11092 (which is herein incorporated by reference) for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. Preparation of DOTMA liposomes is explained in the literature, see, e.g., P. Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417, which is herein incorporated by reference. Similar methods can be used to prepare liposomes from other cationic lipid materials.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed

with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

For example, commercially dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), and dioleoylphosphatidyl ethanolamine (DOPE) can be used in various combinations to make conventional liposomes, with or without the addition of cholesterol. Thus, for example, DOPG/DOPC vesicles can be prepared by drying 50 mg each of DOPG and DOPC under a stream of nitrogen gas into a sonication vial. The sample is placed under a vacuum pump overnight and is hydrated the following day with deionized water. The sample is then sonicated for 2 hours in a capped vial, using a Heat Systems model 350 sonicator equipped with an inverted cup (bath type) probe at the maximum setting while the bath is circulated at 15EC. Alternatively, negatively charged vesicles can be prepared without sonication to produce multilamellar vesicles or by extrusion through nucleopore membranes to produce unilamellar vesicles of discrete size. Other methods are known and available to those of skill in the art.

The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs), with SUVs being preferred. The various liposome-nucleic acid complexes are prepared using methods well known in the art. See, e.g., Straubinger et al., *Methods of Immunology* (1983), 101:512-527, which is herein incorporated by reference. For example, MLVs containing nucleic acid can be prepared by depositing a thin film of phospholipid on the walls of a glass tube and subsequently hydrating with a solution of the material to be encapsulated. SUVs are prepared by extended sonication of MLVs to produce a homogeneous population of unilamellar liposomes. The material to be entrapped is added to a suspension of preformed MLVs and then sonicated. When using liposomes containing cationic lipids, the dried lipid film is resuspended in an appropriate solution such as sterile water or an isotonic buffer solution such as 10 mM Tris/NaCl, sonicated, and then the preformed liposomes are mixed directly with the DNA. The liposome and DNA form a very stable complex due to binding of the positively charged liposomes to the cationic DNA. SUVs find use with small nucleic acid fragments. LUVs are prepared by a number of methods, well known in the art. Commonly used methods include Ca^{2+} -EDTA chelation (Papahadjopoulos et al., *Biochim. Biophys. Acta* (1975) 394:483; Wilson et al., *Cell* (1979) 17:77); ether injection (Deamer, D. and Bangham, A., *Biochim. Biophys. Acta* (1976) 443:629; Ostro et al., *Biochem. Biophys. Res. Commun.* (1977) 76:836; Fraley et al.,

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Proc. Natl. Acad. Sci. USA (1979) 76:3348); detergent dialysis (Enoch, H. and Strittmatter, P., Proc. Natl. Acad. Sci. USA (1979) 76:145); and reverse-phase evaporation (REV) (Fraley et al., J. Biol. Chem. (1980) 255:10431; Szoka, F. and Papahadjopoulos, D., Proc. Natl. Acad. Sci. USA (1978) 75:145; Schaefer-Ridder et al., Science (1982) 215:166), which are
5 herein incorporated by reference.

Generally, the ratio of DNA to liposomes will be from about 10:1 to about 1:10. Preferably, the ration will be from about 5:1 to about 1:5. More preferably, the ration will be about 3:1 to about 1:3. Still more preferably, the ratio will be about 1:1.

U.S. Patent No. 5,676,954 (which is herein incorporated by reference) reports on the
10 injection of genetic material, complexed with cationic liposomes carriers, into mice. U.S. Patent Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication no. WO 94/9469 (which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication no.
15 WO 94/9469 (which are herein incorporated by reference) provide methods for delivering DNA-cationic lipid complexes to mammals.

In certain embodiments, cells are engineered, ex vivo or in vivo, using a retroviral particle containing RNA which comprises a SEQ ID NO:X. Retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine
20 Leukemia Virus, spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are
25 not limited to, the PE501, PA317, R-2, R-AM, PA12, T19-14X, VT-19-17-H2, RCRE, RCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO₄ precipitation. In one
30 alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

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The producer cell line generates infectious retroviral vector particles which include polynucleotide encoding SEQ ID NO:Y. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either in vitro or in vivo. The transduced eukaryotic cells will express SEQ ID NO:Y.

5 In certain other embodiments, cells are engineered, ex vivo or in vivo, with polynucleotide of the present invention contained in an adenovirus vector. Adenovirus can be manipulated such that it encodes and expresses the polypeptide of the present invention, and at the same time is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. Adenovirus expression is achieved without integration of the viral DNA into the
10 host cell chromosome, thereby alleviating concerns about insertional mutagenesis. Furthermore, adenoviruses have been used as live enteric vaccines for many years with an excellent safety profile (Schwartz, A. R. et al. (1974) Am. Rev. Respir. Dis.109:233-238). Finally, adenovirus mediated gene transfer has been demonstrated in a number of instances including transfer of alpha-1-antitrypsin and CFTR to the lungs of cotton rats (Rosenfeld, M.
15 A. et al. (1991) Science 252:431-434; Rosenfeld et al., (1992) Cell 68:143-155). Furthermore, extensive studies to attempt to establish adenovirus as a causative agent in human cancer were uniformly negative (Green, M. et al. (1979) Proc. Natl. Acad. Sci. USA 76:6606).

Suitable adenoviral vectors useful in the present invention are described, for example,
20 in Kozarsky and Wilson, Curr. Opin. Genet. Devel. 3:499-503 (1993); Rosenfeld et al., Cell 68:143-155 (1992); Engelhardt et al., Human Genet. Ther. 4:759-769 (1993); Yang et al., Nature Genet. 7:362-369 (1994); Wilson et al., Nature 365:691-692 (1993); and U.S. Patent No. 5,652,224, which are herein incorporated by reference. For example, the adenovirus vector Ad2 is useful and can be grown in human 293 cells. These cells contain the E1 region
25 of adenovirus and constitutively express Ela and Elb, which complement the defective adenoviruses by providing the products of the genes deleted from the vector. In addition to Ad2, other varieties of adenovirus (e.g., Ad3, Ad5, and Ad7) are also useful in the present invention.

Preferably, the adenoviruses used in the present invention are replication deficient.
30 Replication deficient adenoviruses require the aid of a helper virus and/or packaging cell line to form infectious particles. The resulting virus is capable of infecting cells and can express a polynucleotide of interest which is operably linked to a promoter, but cannot replicate in

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most cells. Replication deficient adenoviruses may be deleted in one or more of all or a portion of the following genes: E1a, E1b, E3, E4, E2a, or L1 through L5.

In certain other embodiments, the cells are engineered, ex vivo or in vivo, using an adeno-associated virus (AAV). AAVs are naturally occurring defective viruses that require helper viruses to produce infectious particles (Muzyczka, N., Curr. Topics in Microbiol. Immunol. 158:97 (1992)). It is also one of the few viruses that may integrate its DNA into non-dividing cells. Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate, but space for exogenous DNA is limited to about 4.5 kb. Methods for producing and using such AAVs are known in the art. See, for example, U.S. Patent Nos. 5,139,941, 5,173,414, 5,354,678, 5,436,146, 5,474,935, 5,478,745, and 5,589,377.

For example, an appropriate AAV vector for use in the present invention will include all the sequences necessary for DNA replication, encapsidation, and host-cell integration. The polynucleotide construct is inserted into the AAV vector using standard cloning methods, such as those found in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (1989). The recombinant AAV vector is then transfected into packaging cells which are infected with a helper virus, using any standard technique, including lipofection, electroporation, calcium phosphate precipitation, etc. Appropriate helper viruses include adenoviruses, cytomegaloviruses, vaccinia viruses, or herpes viruses. Once the packaging cells are transfected and infected, they will produce infectious AAV viral particles which contain the polynucleotide construct. These viral particles are then used to transduce eukaryotic cells, either ex vivo or in vivo. The transduced cells will contain the polynucleotide construct integrated into its genome, and will express the polypeptide of the present invention.

Another method of gene therapy involves operably associating heterologous control regions and endogenous polynucleotide sequences (e.g. encoding the polypeptide of the present invention) via homologous recombination (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not normally expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made, using standard techniques known in the art, which contain the promoter with targeting sequences flanking the promoter. Suitable promoters are described herein. The targeting sequence is sufficiently complementary to an endogenous sequence to permit homologous recombination of the promoter-targeting
5 sequence with the endogenous sequence. The targeting sequence will be sufficiently near the 5' end of the desired endogenous polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination.

The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends.
10 Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter. The amplified promoter and targeting sequences are digested and ligated together.

The promoter-targeting sequence construct is delivered to the cells, either as naked
15 polynucleotide, or in conjunction with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, whole viruses, lipofection, precipitating agents, etc., described in more detail above. The P promoter-targeting sequence can be delivered by any method, included direct needle injection, intravenous injection, topical administration, catheter infusion, particle accelerators, etc. The methods are described in more detail below.

20 The promoter-targeting sequence construct is taken up by cells. Homologous recombination between the construct and the endogenous sequence takes place, such that an endogenous sequence is placed under the control of the promoter. The promoter then drives the expression of the endogenous sequence.

The polynucleotides encoding the polypeptide of the present invention may be
25 administered along with other polynucleotides encoding an angiogenic protein. Examples of angiogenic proteins include, but are not limited to, acidic and basic fibroblast growth factors, VEGF-1, VEGF-2, VEGF-3, epidermal growth factor alpha and beta, platelet-derived endothelial cell growth factor, platelet-derived growth factor, tumor necrosis factor alpha, hepatocyte growth factor, insulin like growth factor, colony stimulating factor, macrophage
30 colony stimulating factor, granulocyte/macrophage colony stimulating factor, and nitric oxide synthase.

Preferably, the polynucleotide encoding the polypeptide of the present invention contains a secretory signal sequence that facilitates secretion of the protein. Typically, the signal sequence is positioned in the coding region of the polynucleotide to be expressed towards or at the 5' end of the coding region. The signal sequence may be
5 homologous or heterologous to the polynucleotide of interest and may be homologous or heterologous to the cells to be transfected. Additionally, the signal sequence may be chemically synthesized using methods known in the art.

Any mode of administration of any of the above-described polynucleotides constructs can be used so long as the mode results in the expression of one or more molecules in an
10 amount sufficient to provide a therapeutic effect. This includes direct needle injection, systemic injection, catheter infusion, biolistic injectors, particle accelerators (i.e., "gene guns"), gelfoam sponge depots, other commercially available depot materials, osmotic pumps (e.g., Alza minipumps), oral or suppository solid (tablet or pill) pharmaceutical formulations, and decanting or topical applications during surgery. For example, direct
15 injection of naked calcium phosphate-precipitated plasmid into rat liver and rat spleen or a protein-coated plasmid into the portal vein has resulted in gene expression of the foreign gene in the rat livers (Kaneda et al., Science 243:375 (1989)).

A preferred method of local administration is by direct injection. Preferably, a recombinant molecule of the present invention complexed with a delivery vehicle is
20 administered by direct injection into or locally within the area of arteries. Administration of a composition locally within the area of arteries refers to injecting the composition centimeters and preferably, millimeters within arteries.

Another method of local administration is to contact a polynucleotide construct of the present invention in or around a surgical wound. For example, a patient can undergo surgery
25 and the polynucleotide construct can be coated on the surface of tissue inside the wound or the construct can be injected into areas of tissue inside the wound.

Therapeutic compositions useful in systemic administration, include recombinant molecules of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise
30 liposomes comprising ligands for targeting the vehicle to a particular site.

Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using

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methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., Proc. Natl. Acad. Sci. USA 189:11277-11281, 1992, which is incorporated herein by reference). Oral delivery can be performed by complexing a polynucleotide construct of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Topical delivery can be performed by mixing a polynucleotide construct of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

Determining an effective amount of substance to be delivered can depend upon a number of factors including, for example, the chemical structure and biological activity of the substance, the age and weight of the animal, the precise condition requiring treatment and its severity, and the route of administration. The frequency of treatments depends upon a number of factors, such as the amount of polynucleotide constructs administered per dose, as well as the health and history of the subject. The precise amount, number of doses, and timing of doses will be determined by the attending physician or veterinarian.

Therapeutic compositions of the present invention can be administered to any animal, preferably to mammals and birds. Preferred mammals include humans, dogs, cats, mice, rats, rabbits sheep, cattle, horses and pigs, with humans being particularly preferred

Biological Activities

Polynucleotides or polypeptides, or agonists or antagonists of the present invention, can be used in assays to test for one or more biological activities. If these polynucleotides or polypeptides, or agonists or antagonists of the present invention, do exhibit activity in a particular assay, it is likely that these molecules may be involved in the diseases associated with the biological activity. Thus, the polynucleotides and polypeptides, and agonists or antagonists could be used to treat the associated disease.

The colon and/or colon cancer related polynucleotides and/or polypeptides of the invention are expressed at significantly enhanced levels in human colon and colon cancer tissues.

Thus, colon and/or colon cancer related polynucleotides and/or polypeptides of the invention may be useful as a therapeutic molecule. It would be useful for diagnosis, detection, treatment and/or prevention of disorders of the colon, including inflammatory

disorders such as, congenital abnormalities, such as atresia and stenosis, Meckel diverticulum, congenital aganglionic megacolon-Hirschsprung disease; enterocolitis, such as diarrhea and dysentery, infectious enterocolitis, including viral gastroenteritis, bacterial enterocolitis, necrotizing enterocolitis, antibiotic-associated colitis (pseudomembranous colitis), and collagenous and lymphocytic colitis, miscellaneous intestinal inflammatory disorders, including parasites and protozoa, amoebic colitis, acquired immunodeficiency syndrome, transplantation, drug-induced intestinal injury, radiation enterocolitis, neutropenic colitis, diverticular colon disease (DCD), inflammatory colonic disease, idiopathic inflammatory bowel disease, such as Crohn's disease (CD), non-inflammatory bowel disease (non-IBD) colonic inflammation; ulcerative disorders such as, ulcerative colitis (UC); eosinophilic colitis; noncancerous tumors, such as, polyps in the colon, adenomas, leiomyomas, lipomas, and angiomas.

Particularly, the colon and/or colon cancer polynucleotides and/or polypeptides of the invention may be a useful therapeutic for tumors, especially of the intestine, such as, carcinoid tumors, lymphomas, non-neoplastic polyps, adenomas, familial syndromes, colorectal carcinogenesis, colorectal carcinoma, cancer of the colon, cancer of the rectum and carcinoid tumors, as well as cancers in other tissues where expression has been indicated. Treatment, diagnosis, detection, and/or prevention of colon disorders could be carried out using a soluble form of a colon and/or colon cancer polypeptides, the colon and/or colon cancer polypeptides ligand, gene therapy, or ex vivo applications. Moreover, inhibitors of colon and/or colon cancer polynucleotides and/or polypeptides, either blocking antibodies or mutant forms, could modulate the expression of colon and/or colon cancer polynucleotides and/or polypeptides. These inhibitors may be useful to treat, diagnose, detect, and/or prevent diseases associated with the misregulation of colon and/or colon cancer polynucleotides and/or polypeptides.

In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells (e.g., colon or colon cancer cells) by administering polypeptides of the invention (e.g., colon and/or colon cancer polypeptides or anti-colon cancer antigen antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell (e.g., a colon cancer cell). In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double

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stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention (e.g., colon or colon cancer polypeptides or anti-colon cancer antigen antibodies) in association with toxins or cytotoxic prodrugs.

By "toxin" is meant compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, cytotoxins (cytotoxic agents), or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNase, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. "Toxin" also includes a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, ^{213}Bi , or other radioisotopes such as, for example, ^{103}Pd , ^{133}Xe , ^{131}I , ^{68}Ge , ^{57}Co , ^{65}Zn , ^{85}Sr , ^{32}P , ^{35}S , ^{90}Y , ^{153}Sm , ^{153}Gd , ^{169}Yb , ^{51}Cr , ^{54}Mn , ^{75}Se , ^{113}Sn , $^{90}\text{Yttrium}$, ^{117}Tin , $^{186}\text{Rhenium}$, $^{166}\text{Holmium}$, and $^{188}\text{Rhenium}$; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

Techniques known in the art may be applied to label antibodies of the invention. Such techniques include, but are not limited to, the use of bifunctional conjugating agents (see e.g., U.S. Patent Nos. 5,756,065; 5,714,631; 5,696,239; 5,652,361; 5,505,931; 5,489,425; 5,435,990; 5,428,139; 5,342,604; 5,274,119; 4,994,560; and 5,808,003; the contents of each of which are hereby incorporated by reference in its entirety). A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g.,

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methotrexate, 6-mercaptopurine, 6- thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis- dichlorodiamine platinum (II) (DDP) cisplatin),
5 anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

By "cytotoxic prodrug" is meant a non-toxic compound that is converted by an enzyme, normally present in the cell, into a cytotoxic compound. Cytotoxic prodrugs that
10 may be used according to the methods of the invention include, but are not limited to, glutamyl derivatives of benzoic acid mustard alkylating agent, phosphate derivatives of etoposide or mitomycin C, cytosine arabinoside, daunorubisin, and phenoxyacetamide derivatives of doxorubicin.

It will be appreciated that conditions caused by a decrease in the standard or normal
15 level of colon and/or colon cancer polynucleotide and/or polypeptide activity in an individual, particularly disorders of the colon, can be treated by administration of colon or colon cancer polypeptide (e.g., in the form of soluble extracellular domain or cells expressing the complete protein) or agonist. Thus, the invention also provides a method of treatment of an individual in need of an increased level of PSGR activity comprising administering to
20 such an individual a pharmaceutical composition comprising an amount of an isolated colon or colon cancer polypeptide of the invention, or agonist thereof (e.g, an agonistic anti-colon cancer antigen antibody), effective to increase the colon and/or colon cancer polypeptide activity level in such an individual.

It will also be appreciated that conditions caused by a increase in the standard or normal level
25 of colon and/or colon cancer polynucleotides and/or polypeptides activity in an individual, particularly disorders of the colon, can be treated by administration of colon or colon cancer related polypeptides (e.g., in the form of soluble extracellular domain or cells expressing the complete protein) or antagonist (e.g., an antagonistic anti-colon cancer antigen antibody). Thus, the invention also provides a method of treatment of an individual in need of an
30 decreased level of colon and/or colon cancer polynucleotides and/or polypeptides activity comprising administering to such an individual a pharmaceutical composition comprising an amount of an isolated colon polypeptide of the invention, or antagonist thereof, effective to

decrease the colon and/or colon cancer polynucleotides and/or polypeptides activity level in such an individual.

5 **Immune Activity**

A polypeptide or polynucleotide, or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing deficiencies, diseases, or disorders and/or conditions of the immune system, by, for example, activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells
10 develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune deficiencies or disorders may be genetic, somatic, such as cancer or some autoimmune disorders, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, polynucleotides or polypeptides, or
15 agonists or antagonists of the present invention can be used as a marker or detector of a particular immune system disease or disorder.

Polynucleotides or polypeptides, or agonists or antagonists of the present invention may be useful in treating, preventing, detecting and/or diagnosing diseases, deficiencies or disorders and/or conditions of hematopoietic cells. Polynucleotides or polypeptides, or
20 agonists or antagonists of the present invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat those disorders associated with a decrease in certain (or many) types hematopoietic cells. Examples of immunologic deficiency syndromes include, but are not limited to: blood protein disorders (e.g. agammaglobulinemia, dysgammaglobulinemia), ataxia telangiectasia,
25 common variable immunodeficiency, Digeorge Syndrome, HIV infection, HTLV-BLV infection, leukocyte adhesion deficiency syndrome, lymphopenia, phagocyte bactericidal dysfunction, severe combined immunodeficiency (SCIDs), Wiskott-Aldrich Disorder, anemia, thrombocytopenia, or hemoglobinuria.

Moreover, polynucleotides or polypeptides, or agonists or antagonists of the present
30 invention could also be used to modulate hemostatic (the stopping of bleeding) or thrombolytic activity (clot formation). For example, by increasing hemostatic or thrombolytic activity, polynucleotides or polypeptides, or agonists or antagonists of the

present invention could be used to treat blood coagulation disorders (e.g., afibrinogenemia, factor deficiencies), blood platelet disorders (e.g. thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, polynucleotides or polypeptides, or agonists or antagonists of the present invention that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting. These molecules could be important in the treatment of heart attacks (infarction), strokes, or scarring.

Polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be useful in treating, preventing, detecting and/or diagnosing autoimmune disorders. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of polynucleotides or polypeptides, or agonists or antagonists of the present invention that can inhibit an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune disorders.

Autoimmune diseases or disorders that may be treated, prevented, and/or diagnosed by polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention include, but are not limited to, one or more of the following: autoimmune hemolytic anemia, autoimmune neonatal thrombocytopenia, idiopathic thrombocytopenia purpura, autoimmune thrombocytopenia, hemolytic anemia, antiphospholipid syndrome, dermatitis, allergic encephalomyelitis, myocarditis, relapsing polychondritis, rheumatic heart disease, glomerulonephritis (e.g. IgA nephropathy), Multiple Sclerosis, Neuritis, Uveitis Ophthalmia, Polyendocrinopathies, Purpura (e.g., Henloch-Schoenlein purpura), Reiter's Disease, Stiff-Man Syndrome, Autoimmune Pulmonary Inflammation, Autism, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye, autoimmune thyroiditis, hypothyroidism (i.e., Hashimoto's thyroiditis, systemic lupus erythematosus, Goodpasture's syndrome, Pemphigus, Receptor autoimmunities such as, for example, (a) Graves' Disease, (b) Myasthenia Gravis, and (c) insulin resistance, autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura, rheumatoid arthritis, scleroderma with anti-collagen antibodies, mixed connective tissue disease, polymyositis/dermatomyositis, pernicious anemia, idiopathic Addison's disease, infertility, glomerulonephritis such as primary glomerulonephritis and IgA nephropathy, bullous pemphigoid, Sjogren's syndrome, diabetes mellitus, and adrenergic drug resistance (including adrenergic drug resistance with

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asthma or cystic fibrosis), chronic active hepatitis, primary biliary cirrhosis, other endocrine gland failure, vitiligo, vasculitis, post-MI, cardiomy syndrome, urticaria, atopic dermatitis, asthma, inflammatory myopathies, and other inflammatory, granulomatous, degenerative, and atrophic disorders.

5 Additional autoimmune disorders (that are probable) that may be treated, prevented, and/or diagnosed with the compositions of the invention include, but are not limited to, rheumatoid arthritis (often characterized, e.g., by immune complexes in joints), scleroderma with anti-collagen antibodies (often characterized, e.g., by nucleolar and other nuclear antibodies), mixed connective tissue disease (often characterized, e.g., by antibodies to
10 extractable nuclear antigens (e.g., ribonucleoprotein)), polymyositis (often characterized, e.g., by nonhistone ANA), pernicious anemia (often characterized, e.g., by antiparietal cell, microsomes, and intrinsic factor antibodies), idiopathic Addison's disease (often characterized, e.g., by humoral and cell-mediated adrenal cytotoxicity, infertility (often characterized, e.g., by antispermatozoal antibodies), glomerulonephritis (often characterized,
15 e.g., by glomerular basement membrane antibodies or immune complexes), bullous pemphigoid (often characterized, e.g., by IgG and complement in basement membrane), Sjogren's syndrome (often characterized, e.g., by multiple tissue antibodies, and/or a specific nonhistone ANA (SS-B)), diabetes mellitus (often characterized, e.g., by cell-mediated and humoral islet cell antibodies), and adrenergic drug resistance (including adrenergic drug
20 resistance with asthma or cystic fibrosis) (often characterized, e.g., by beta-adrenergic receptor antibodies).

Additional autoimmune disorders (that are possible) that may be treated, prevented, and/or diagnosed with the compositions of the invention include, but are not limited to, chronic active hepatitis (often characterized, e.g., by smooth muscle antibodies), primary
25 biliary cirrhosis (often characterized, e.g., by mitochondrial antibodies), other endocrine gland failure (often characterized, e.g., by specific tissue antibodies in some cases), vitiligo (often characterized, e.g., by melanocyte antibodies), vasculitis (often characterized, e.g., by Ig and complement in vessel walls and/or low serum complement), post-MI (often characterized, e.g., by myocardial antibodies), cardiomy syndrome (often characterized, e.g., by
30 myocardial antibodies), urticaria (often characterized, e.g., by IgG and IgM antibodies to IgE), atopic dermatitis (often characterized, e.g., by IgG and IgM antibodies to IgE), asthma

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(often characterized, e.g., by IgG and IgM antibodies to IgE), and many other inflammatory, granulomatous, degenerative, and atrophic disorders.

In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prevented, and/or
5 diagnosed using for example, antagonists or agonists, polypeptides or polynucleotides, or antibodies of the present invention.

In a preferred embodiment polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could be used as an agent to boost immunoresponsiveness among B cell and/or T cell immunodeficient individuals.

10 B cell immunodeficiencies that may be ameliorated or treated by administering the polypeptides or polynucleotides of the invention, and/or agonists thereof, include, but are not limited to, severe combined immunodeficiency (SCID)-X linked, SCID-autosomal, adenosine deaminase deficiency (ADA deficiency), X-linked agammaglobulinemia (XLA), Bruton's disease, congenital agammaglobulinemia, X-linked infantile agammaglobulinemia,
15 acquired agammaglobulinemia, adult onset agammaglobulinemia, late-onset agammaglobulinemia, dysgammaglobulinemia, hypogammaglobulinemia, transient hypogammaglobulinemia of infancy, unspecified hypogammaglobulinemia, agammaglobulinemia, common variable immunodeficiency (CVI) (acquired), Wiskott-Aldrich Syndrome (WAS), X-linked immunodeficiency with hyper IgM, non X-linked
20 immunodeficiency with hyper IgM, selective IgA deficiency, IgG subclass deficiency (with or without IgA deficiency), antibody deficiency with normal or elevated Igs, immunodeficiency with thymoma, Ig heavy chain deletions, kappa chain deficiency, B cell lymphoproliferative disorder (BLPD), selective IgM immunodeficiency, recessive agammaglobulinemia (Swiss type), reticular dysgenesis, neonatal neutropenia, severe
25 congenital leukopenia, thymic aplasia or dysplasia with immunodeficiency, ataxia-telangiectasia, short limbed dwarfism, X-linked lymphoproliferative syndrome (XLP), Nezelof syndrome-combined immunodeficiency with Igs, purine nucleoside phosphorylase deficiency (PNP), MHC Class II deficiency (Bare Lymphocyte Syndrome) and severe combined immunodeficiency.

30 T cell deficiencies that may be ameliorated or treated by administering the polypeptides or polynucleotides of the invention, and/or agonists thereof include, but are not limited to, for example, DiGeorge anomaly, thymic hypoplasia, third and fourth pharyngeal

pouch syndrome, 22q11.2 deletion, chronic mucocutaneous candidiasis, natural killer cell deficiency (NK), idiopathic CD4+ T-lymphocytopenia, immunodeficiency with predominant T cell defect (unspecified), and unspecified immunodeficiency of cell mediated immunity. In specific embodiments, DiGeorge anomaly or conditions associated with DiGeorge anomaly are ameliorated or treated by, for example, administering the polypeptides or polynucleotides of the invention, or antagonists or agonists thereof.

Other immunodeficiencies that may be ameliorated or treated by administering polypeptides or polynucleotides of the invention, and/or agonists thereof, include, but are not limited to, severe combined immunodeficiency (SCID; e.g., X-linked SCID, autosomal SCID, and adenosine deaminase deficiency), ataxia-telangiectasia, Wiskott-Aldrich syndrome, short-limber dwarfism, X-linked lymphoproliferative syndrome (XLP), Nezelof syndrome (e.g., purine nucleoside phosphorylase deficiency), MHC Class II deficiency. In specific embodiments, ataxia-telangiectasia or conditions associated with ataxia-telangiectasia are ameliorated or treated by administering the polypeptides or polynucleotides of the invention, and/or agonists thereof.

In a specific preferred embodiment, rheumatoid arthritis is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. In another specific preferred embodiment, systemic lupus erythematosus is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. In another specific preferred embodiment, idiopathic thrombocytopenia purpura is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. In another specific preferred embodiment IgA nephropathy is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prevented, and/or diagnosed using antibodies against the protein of the invention.

Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated, prevented, and/or diagnosed using polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof. Moreover, these molecules can be used to treat, prevent, and/or diagnose anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

Moreover, inflammatory conditions may also be treated, diagnosed, and/or prevented with polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. Such inflammatory conditions include, but are not limited to, for example, respiratory disorders (such as, e.g., asthma and allergy); gastrointestinal disorders (such as, e.g., inflammatory bowel disease); cancers (such as, e.g., gastric, ovarian, lung, bladder, liver, and breast); CNS disorders (such as, e.g., multiple sclerosis, blood-brain barrier permeability, ischemic brain injury and/or stroke, traumatic brain injury, neurodegenerative disorders (such as, e.g., Parkinson's disease and Alzheimer's disease), AIDS-related dementia, and prion disease); cardiovascular disorders (such as, e.g., atherosclerosis, myocarditis, cardiovascular disease, and cardiopulmonary bypass complications); as well as many additional diseases, conditions, and disorders that are characterized by inflammation (such as, e.g., chronic hepatitis (B and C), rheumatoid arthritis, gout, trauma, septic shock, pancreatitis, sarcoidosis, dermatitis, renal ischemia-reperfusion injury, Grave's disease, systemic lupus erythematosus, diabetes mellitus (i.e., type 1 diabetes), and allogenic transplant rejection).

In specific embodiments, polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, are useful to treat, diagnose, and/or prevent transplantation rejections, graft-versus-host disease, autoimmune and inflammatory diseases (e.g., immune complex-induced vasculitis, glomerulonephritis, hemolytic anemia, myasthenia gravis, type II collagen-induced arthritis, experimental allergic and hyperacute xenograft rejection, rheumatoid arthritis, and systemic lupus erythematosus (SLE). Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. Polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, that inhibit an immune response, particularly the activation, proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

Similarly, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may also be used to modulate and/or diagnose inflammation. For example, since polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists of the invention may inhibit the activation, proliferation and/or differentiation of cells involved in an inflammatory response, these molecules can be used to treat, diagnose,

or prognose, inflammatory conditions, both chronic and acute conditions, including, but not limited to, inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, and resulting from over
5 production of cytokines (e.g., TNF or IL-1).

Polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the invention can be used to treat, detect, and/or prevent infectious agents. For example, by increasing the immune response, particularly increasing the proliferation activation and/or
10 differentiation of B and/or T cells, infectious diseases may be treated, detected, and/or prevented. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may also directly inhibit the infectious agent (refer to section of application listing infectious agents,
15 etc), without necessarily eliciting an immune response.

Additional preferred embodiments of the invention include, but are not limited to, the use of polypeptides, antibodies, polynucleotides and/or agonists or antagonists in the following applications:

Administration to an animal (e.g., mouse, rat, rabbit, hamster, guinea pig, pigs, micro-
20 pig, chicken, camel, goat, horse, cow, sheep, dog, cat, non-human primate, and human, most preferably human) to boost the immune system to produce increased quantities of one or more antibodies (e.g., IgG, IgA, IgM, and IgE), to induce higher affinity antibody production (e.g., IgG, IgA, IgM, and IgE), and/or to increase an immune response.

Administration to an animal (including, but not limited to, those listed above, and also
25 including transgenic animals) incapable of producing functional endogenous antibody molecules or having an otherwise compromised endogenous immune system, but which is capable of producing human immunoglobulin molecules by means of a reconstituted or partially reconstituted immune system from another animal (see, e.g., published PCT Application Nos. WO98/24893, WO/9634096, WO/9633735, and WO/9110741.

30 A vaccine adjuvant that enhances immune responsiveness to specific antigen.

An adjuvant to enhance tumor-specific immune responses.

An adjuvant to enhance anti-viral immune responses. Anti-viral immune responses that may be enhanced using the compositions of the invention as an adjuvant, include virus and virus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: AIDS, meningitis, Dengue, EBV, and hepatitis (e.g., hepatitis B). In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: HIV/AIDS, Respiratory syncytial virus, Dengue, Rotavirus, Japanese B encephalitis, Influenza A and B, Parainfluenza, Measles, Cytomegalovirus, Rabies, Junin, Chikungunya, Rift Valley fever, Herpes simplex, and yellow fever.

An adjuvant to enhance anti-bacterial or anti-fungal immune responses. Anti-bacterial or anti-fungal immune responses that may be enhanced using the compositions of the invention as an adjuvant, include bacteria or fungus and bacteria or fungus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: tetanus, Diphtheria, botulism, and meningitis type B. In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: *Vibrio cholerae*, *Mycobacterium leprae*, *Salmonella typhi*, *Salmonella paratyphi*, *Meisseria meningitidis*, *Streptococcus pneumoniae*, Group B streptococcus, *Shigella spp.*, Enterotoxigenic *Escherichia coli*, Enterohemorrhagic *E. coli*, *Borrelia burgdorferi*, and Plasmodium (malaria).

An adjuvant to enhance anti-parasitic immune responses. Anti-parasitic immune responses that may be enhanced using the compositions of the invention as an adjuvant, include parasite and parasite associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a parasite. In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to Plasmodium (malaria).

As a stimulator of B cell responsiveness to pathogens.

As an activator of T cells.

As an agent that elevates the immune status of an individual prior to their receipt of immunosuppressive therapies.

As an agent to induce higher affinity antibodies.

5 As an agent to increase serum immunoglobulin concentrations.

As an agent to accelerate recovery of immunocompromised individuals.

As an agent to boost immunoresponsiveness among aged populations.

As an immune system enhancer prior to, during, or after bone marrow transplant and/or other transplants (e.g., allogeneic or xenogeneic organ transplantation). With respect
10 to transplantation, compositions of the invention may be administered prior to, concomitant with, and/or after transplantation. In a specific embodiment, compositions of the invention are administered after transplantation, prior to the beginning of recovery of T-cell populations. In another specific embodiment, compositions of the invention are first administered after transplantation after the beginning of recovery of T cell populations, but
15 prior to full recovery of B cell populations.

As an agent to boost immunoresponsiveness among individuals having an acquired loss of B cell function. Conditions resulting in an acquired loss of B cell function that may be ameliorated or treated by administering the polypeptides, antibodies, polynucleotides and/or agonists or antagonists thereof, include, but are not limited to, HIV Infection, AIDS,
20 bone marrow transplant, and B cell chronic lymphocytic leukemia (CLL).

As an agent to boost immunoresponsiveness among individuals having a temporary immune deficiency. Conditions resulting in a temporary immune deficiency that may be ameliorated or treated by administering the polypeptides, antibodies, polynucleotides and/or agonists or antagonists thereof, include, but are not limited to, recovery from viral infections
25 (e.g., influenza), conditions associated with malnutrition, recovery from infectious mononucleosis, or conditions associated with stress, recovery from measles, recovery from blood transfusion, recovery from surgery.

As a regulator of antigen presentation by monocytes, dendritic cells, and/or B-cells. In one embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists
30 of the present invention enhance antigen presentation or antagonizes antigen presentation in vitro or in vivo. Moreover, in related embodiments, said enhancement or antagonization of

antigen presentation may be useful as an anti- tumor treatment or to modulate the immune system.

As an agent to direct an individuals immune system towards development of a humoral response (i.e. TH2) as opposed to a TH1 cellular response.

5 As a means to induce tumor proliferation and thus make it more susceptible to anti-neoplastic agents. For example, multiple myeloma is a slowly dividing disease and is thus refractory to virtually all anti-neoplastic regimens. If these cells were forced to proliferate more rapidly their susceptibility profile would likely change.

As a stimulator of B cell production in pathologies such as AIDS, chronic lymphocyte disorder and/or Common Variable Immunodeficiency.

As a therapy for generation and/or regeneration of lymphoid tissues following surgery, trauma or genetic defect.

As a gene-based therapy for genetically inherited disorders resulting in immuno-incompetence such as observed among SCID patients.

15 As an antigen for the generation of antibodies to inhibit or enhance immune mediated responses against polypeptides of the invention.

As a means of activating T cells.

As a means of activating monocytes/macrophages to defend against parasitic diseases that effect monocytes such as Leshmania.

20 As pretreatment of bone marrow samples prior to transplant. Such treatment would increase B cell representation and thus accelerate recover.

As a means of regulating secreted cytokines that are elicited by polypeptides of the invention.

25 Additionally, polypeptides or polynucleotides of the invention, and/or agonists thereof, may be used to treat or prevent IgE-mediated allergic reactions. Such allergic reactions include, but are not limited to, asthma, rhinitis, and eczema.

All of the above described applications as they may apply to veterinary medicine.

Antagonists of the invention include, for example, binding and/or inhibitory antibodies, antisense nucleic acids, or ribozymes. These would be expected to reverse many of the activities of the ligand described above as well as find clinical or practical application as:

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A means of blocking various aspects of immune responses to foreign agents or self. Examples include autoimmune disorders such as lupus, and arthritis, as well as immunoresponsiveness to skin allergies, inflammation, bowel disease, injury and pathogens.

5 A therapy for preventing the B cell proliferation and Ig secretion associated with autoimmune diseases such as idiopathic thrombocytopenic purpura, systemic lupus erythramatosus and MS.

An inhibitor of B and/or T cell migration in endothelial cells. This activity disrupts tissue architecture or cognate responses and is useful, for example in disrupting immune responses, and blocking sepsis.

10 An inhibitor of graft versus host disease or transplant rejection.

A therapy for B cell and/or T cell malignancies such as ALL, Hodgkins disease, non-Hodgkins lymphoma, Chronic lymphocyte leukemia, plasmacytomas, multiple myeloma, Burkitt's lymphoma, and EBV-transformed diseases.

15 A therapy for chronic hypergammaglobulinemia evident in such diseases as monoclonalgammopathy of undetermined significance (MGUS), Waldenstrom's disease, related idiopathic monoclonalgammopathies, and plasmacytomas.

A therapy for decreasing cellular proliferation of Large B-cell Lymphomas.

A means of decreasing the involvement of B cells and Ig associated with Chronic Myelogenous Leukemia.

20 An immunosuppressive agent(s).

Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to modulate IgE concentrations in vitro or in vivo.

25 In another embodiment, administration of polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the invention, may be used to treat or prevent IgE-mediated allergic reactions including, but not limited to, asthma, rhinitis, and eczema.

The agonists and antagonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as described herein.

30 The agonists or antagonists may be employed for instance to inhibit polypeptide chemotaxis and activation of macrophages and their precursors, and of neutrophils, basophils, B lymphocytes and some T-cell subsets, e.g., activated and CD8 cytotoxic T cells and natural killer cells, in certain auto-immune and chronic inflammatory and infective diseases. Examples of autoimmune diseases are described herein and include multiple sclerosis, and

insulin-dependent diabetes. The antagonists or agonists may also be employed to treat infectious diseases including silicosis, sarcoidosis, idiopathic pulmonary fibrosis by, for example, preventing the recruitment and activation of mononuclear phagocytes. They may also be employed to treat idiopathic hyper-eosinophilic syndrome by, for example, preventing eosinophil production and migration. The antagonists or agonists or may also be employed for treating atherosclerosis, for example, by preventing monocyte infiltration in the artery wall.

Antibodies against polypeptides of the invention may be employed to treat ARDS.

Agonists and/or antagonists of the invention also have uses in stimulating wound and tissue repair, stimulating angiogenesis, stimulating the repair of vascular or lymphatic diseases or disorders. Additionally, agonists and antagonists of the invention may be used to stimulate the regeneration of mucosal surfaces.

In a specific embodiment, polynucleotides or polypeptides, and/or agonists thereof are used to treat or prevent a disorder characterized by primary or acquired immunodeficiency, deficient serum immunoglobulin production, recurrent infections, and/or immune system dysfunction. Moreover, polynucleotides or polypeptides, and/or agonists thereof may be used to treat or prevent infections of the joints, bones, skin, and/or parotid glands, blood-borne infections (e.g., sepsis, meningitis, septic arthritis, and/or osteomyelitis), autoimmune diseases (e.g., those disclosed herein), inflammatory disorders, and malignancies, and/or any disease or disorder or condition associated with these infections, diseases, disorders and/or malignancies) including, but not limited to, CVID, other primary immune deficiencies, HIV disease, CLL, recurrent bronchitis, sinusitis, otitis media, conjunctivitis, pneumonia, hepatitis, meningitis, herpes zoster (e.g., severe herpes zoster), and/or pneumocystis carinii.

In another embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention are used to treat, and/or diagnose an individual having common variable immunodeficiency disease ("CVID"; also known as "acquired agammaglobulinemia" and "acquired hypogammaglobulinemia") or a subset of this disease.

In a specific embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to treat, diagnose, and/or prevent (1) cancers or neoplasms and (2) autoimmune cell or tissue-related cancers or neoplasms. In a preferred embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or

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antagonists of the present invention conjugated to a toxin or a radioactive isotope, as described herein, may be used to treat, diagnose, and/or prevent acute myelogeneous leukemia. In a further preferred embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention conjugated to a toxin or a radioactive isotope, as described herein, may be used to treat, diagnose, and/or prevent, chronic myelogeneous leukemia, multiple myeloma, non-Hodgkins lymphoma, and/or Hodgkins disease.

In another specific embodiment, polynucleotides or polypeptides, and/or agonists or antagonists of the invention may be used to treat, diagnose, prognose, and/or prevent selective IgA deficiency, myeloperoxidase deficiency, C2 deficiency, ataxia-telangiectasia, DiGeorge anomaly, common variable immunodeficiency (CVI), X-linked agammaglobulinemia, severe combined immunodeficiency (SCID), chronic granulomatous disease (CGD), and Wiskott-Aldrich syndrome.

Examples of autoimmune disorders that can be treated or detected are described above and also include, but are not limited to: Addison's Disease, hemolytic anemia, antiphospholipid syndrome, rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture's Syndrome, Graves' Disease, Multiple Sclerosis, Myasthenia Gravis, Neuritis, Ophthalmia, Bullous Pemphigoid, Pemphigus, Polyendocrinopathies, Purpura, Reiter's Disease, Stiff-Man Syndrome, Autoimmune Thyroiditis, Systemic Lupus Erythematosus, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitis, and autoimmune inflammatory eye disease.

Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated by polynucleotides or polypeptides, or agonists or antagonists of the present invention. Moreover, these molecules can be used to treat anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

Polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be used to treat and/or prevent organ rejection or graft-versus-host disease (GVHD). Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. The administration of

polynucleotides or polypeptides, or agonists or antagonists of the present invention that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

Similarly, polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be used to modulate inflammation. For example, polynucleotides or polypeptides, or agonists or antagonists of the present invention may inhibit the proliferation and differentiation of cells involved in an inflammatory response. These molecules can be used to treat inflammatory conditions, both chronic and acute conditions, including chronic prostatitis, granulomatous prostatitis and malacoplakia, inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, or resulting from over production of cytokines (e.g., TNF or IL-1.)

In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prognosed, prevented, and/or diagnosed using antibodies against the polypeptide of the invention.

As an agent to boost immunoresponsiveness among B cell immunodeficient individuals, such as, for example, an individual who has undergone a partial or complete splenectomy.

Additionally, polynucleotides, polypeptides, and/or antagonists of the invention may affect apoptosis, and therefore, would be useful in treating a number of diseases associated with increased cell survival or the inhibition of apoptosis. For example, diseases associated with increased cell survival or the inhibition of apoptosis that could be treated or detected by polynucleotides, polypeptides, and/or antagonists of the invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis

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and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection. In preferred embodiments, polynucleotides, polypeptides, and/or antagonists of the invention are used to inhibit growth, progression, and/or metastasis of cancers, in particular those listed above.

Additional diseases or conditions associated with increased cell survival that could be treated or detected by polynucleotides, polypeptides, and/or antagonists of the invention, include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma.

Diseases associated with increased apoptosis that could be treated or detected by polynucleotides, polypeptides, and/or antagonists of the invention, include AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome,

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Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestosis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

Hyperproliferative diseases and/or disorders that could be detected and/or treated by polynucleotides, polypeptides, and/or antagonists of the invention, include, but are not limited to neoplasms located in the: liver, abdomen, bone, breast, digestive system, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

Similarly, other hyperproliferative disorders can also be treated or detected by polynucleotides, polypeptides, and/or antagonists of the invention. Examples of such hyperproliferative disorders include, but are not limited to: hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenström's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

20

Hyperproliferative Disorders

Polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used to treat or detect hyperproliferative disorders, including neoplasms. Polynucleotides or polypeptides, or agonists or antagonists of the present invention may inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, Polynucleotides or polypeptides, or agonists or antagonists of the present invention may proliferate other cells which can inhibit the hyperproliferative disorder.

For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative disorders can be treated. This immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response.

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Alternatively, decreasing an immune response may also be a method of treating hyperproliferative disorders, such as a chemotherapeutic agent.

Examples of hyperproliferative disorders that can be treated or detected by Polynucleotides or polypeptides, or agonists or antagonists of the present invention include, but are not limited to neoplasms located in the: colon, abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

Similarly, other hyperproliferative disorders can also be treated or detected by polynucleotides or polypeptides, or agonists or antagonists of the present invention. Examples of such hyperproliferative disorders include, but are not limited to: hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenström's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

One preferred embodiment utilizes polynucleotides of the present invention to inhibit aberrant cellular division, by gene therapy using the present invention, and/or protein fusions or fragments thereof.

Thus, the present invention provides a method for treating cell proliferative disorders by inserting into an abnormally proliferating cell a polynucleotide of the present invention, wherein said polynucleotide represses said expression.

Another embodiment of the present invention provides a method of treating cell-proliferative disorders in individuals comprising administration of one or more active gene copies of the present invention to an abnormally proliferating cell or cells. In a preferred embodiment, polynucleotides of the present invention is a DNA construct comprising a recombinant expression vector effective in expressing a DNA sequence encoding said polynucleotides. In another preferred embodiment of the present invention, the DNA construct encoding the polynucleotides of the present invention is inserted into cells to be treated utilizing a retrovirus, or more preferably an adenoviral vector (See G J. Nabel, et. al., PNAS 1999 96: 324-326, which is hereby incorporated by reference). In a most preferred embodiment, the viral vector is defective and will not transform non-proliferating cells, only proliferating cells. Moreover, in a preferred embodiment, the polynucleotides of the present

invention inserted into proliferating cells either alone, or in combination with or fused to other polynucleotides, can then be modulated via an external stimulus (i.e. magnetic, specific small molecule, chemical, or drug administration, etc.), which acts upon the promoter upstream of said polynucleotides to induce expression of the encoded protein product. As such the beneficial therapeutic affect of the present invention may be expressly
5 modulated (i.e. to increase, decrease, or inhibit expression of the present invention) based upon said external stimulus.

Polynucleotides of the present invention may be useful in repressing expression of oncogenic genes or antigens. By "repressing expression of the oncogenic genes " is intended
10 the suppression of the transcription of the gene, the degradation of the gene transcript (pre-message RNA), the inhibition of splicing, the destruction of the messenger RNA, the prevention of the post-translational modifications of the protein, the destruction of the protein, or the inhibition of the normal function of the protein.

For local administration to abnormally proliferating cells, polynucleotides of the present invention may be administered by any method known to those of skill in the art including, but not limited to transfection, electroporation, microinjection of cells, or in
15 vehicles such as liposomes, lipofectin, or as naked polynucleotides, or any other method described throughout the specification. The polynucleotide of the present invention may be delivered by known gene delivery systems such as, but not limited to, retroviral vectors
20 (Gilboa, J. Virology 44:845 (1982); Hocke, Nature 320:275 (1986); Wilson, et al., Proc. Natl. Acad. Sci. U.S.A. 85:3014), vaccinia virus system (Chakrabarty et al., Mol. Cell Biol. 5:3403 (1985) or other efficient DNA delivery systems (Yates et al., Nature 313:812 (1985)) known to those skilled in the art. These references are exemplary only and are hereby incorporated by reference. In order to specifically deliver or transfect cells which are abnormally
25 proliferating and spare non-dividing cells, it is preferable to utilize a retrovirus, or adenoviral (as described in the art and elsewhere herein) delivery system known to those of skill in the art. Since host DNA replication is required for retroviral DNA to integrate and the retrovirus will be unable to self replicate due to the lack of the retrovirus genes needed for its life cycle. Utilizing such a retroviral delivery system for polynucleotides of the present invention will
30 target said gene and constructs to abnormally proliferating cells and will spare the non-dividing normal cells.

The polynucleotides of the present invention may be delivered directly to cell proliferative disorder/disease sites in internal organs, body cavities and the like by use of imaging devices used to guide an injecting needle directly to the disease site. The polynucleotides of the present invention may also be administered to disease sites at the time of surgical intervention.

By "cell proliferative disease" is meant any human or animal disease or disorder, affecting any one or any combination of organs, cavities, or body parts, which is characterized by single or multiple local abnormal proliferations of cells, groups of cells, or tissues, whether benign or malignant.

Any amount of the polynucleotides of the present invention may be administered as long as it has a biologically inhibiting effect on the proliferation of the treated cells. Moreover, it is possible to administer more than one of the polynucleotide of the present invention simultaneously to the same site. By "biologically inhibiting" is meant partial or total growth inhibition as well as decreases in the rate of proliferation or growth of the cells. The biologically inhibitory dose may be determined by assessing the effects of the polynucleotides of the present invention on target malignant or abnormally proliferating cell growth in tissue culture, tumor growth in animals and cell cultures, or any other method known to one of ordinary skill in the art.

The present invention is further directed to antibody-based therapies which involve administering of anti-polypeptides and anti-polynucleotide antibodies to a mammalian, preferably human, patient for treating one or more of the described disorders. Methods for producing anti-polypeptides and anti-polynucleotide antibodies polyclonal and monoclonal antibodies are described in detail elsewhere herein. Such antibodies may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

In particular, the antibodies, fragments and derivatives of the present invention are useful for treating a subject having or developing cell proliferative and/or differentiation disorders as described herein. Such treatment comprises administering a single or multiple doses of the antibody, or a fragment, derivative, or a conjugate thereof.

5 The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors, for example., which serve to increase the number or activity of effector cells which interact with the antibodies.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing
10 antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides, including fragments thereof. Preferred binding affinities include those with a
15 dissociation constant or K_d less than $5 \times 10^{-6}M$, $10^{-6}M$, $5 \times 10^{-7}M$, $10^{-7}M$, $5 \times 10^{-8}M$, $10^{-8}M$, $5 \times 10^{-9}M$, $10^{-9}M$, $5 \times 10^{-10}M$, $10^{-10}M$, $5 \times 10^{-11}M$, $10^{-11}M$, $5 \times 10^{-12}M$, $10^{-12}M$, $5 \times 10^{-13}M$, $10^{-13}M$, $5 \times 10^{-14}M$, $10^{-14}M$, $5 \times 10^{-15}M$, and $10^{-15}M$.

Moreover, polypeptides of the present invention are useful in inhibiting the angiogenesis of proliferative cells or tissues, either alone, as a protein fusion, or in combination with other
20 polypeptides directly or indirectly, as described elsewhere herein. In a most preferred embodiment, said anti-angiogenesis effect may be achieved indirectly, for example, through the inhibition of hematopoietic, tumor-specific cells, such as tumor-associated macrophages (See Joseph IB, et al. J Natl Cancer Inst, 90(21):1648-53 (1998), which is hereby incorporated by reference). Antibodies directed to polypeptides or polynucleotides of the
25 present invention may also result in inhibition of angiogenesis directly, or indirectly (See Witte L, et al., Cancer Metastasis Rev. 17(2):155-61 (1998), which is hereby incorporated by reference)).

Polypeptides, including protein fusions, of the present invention, or fragments thereof may be useful in inhibiting proliferative cells or tissues through the induction of apoptosis.
30 Said polypeptides may act either directly, or indirectly to induce apoptosis of proliferative cells and tissues, for example in the activation of a death-domain receptor, such as tumor necrosis factor (TNF) receptor-1, CD95 (Fas/APO-1), TNF-receptor-related apoptosis-

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mediated protein (TRAMP) and TNF-related apoptosis-inducing ligand (TRAIL) receptor-1 and -2 (See Schulze-Osthoff K, et.al., Eur J Biochem 254(3):439-59 (1998), which is hereby incorporated by reference). Moreover, in another preferred embodiment of the present invention, said polypeptides may induce apoptosis through other mechanisms, such as in the
5 activation of other proteins which will activate apoptosis, or through stimulating the expression of said proteins, either alone or in combination with small molecule drugs or adjuvants, such as apoptonin, galectins, thioredoxins, antiinflammatory proteins (See for example, Mutat Res 400(1-2):447-55 (1998), Med Hypotheses.50(5):423-33 (1998), Chem Biol Interact. Apr 24;111-112:23-34 (1998), J Mol Med.76(6):402-12 (1998), Int J Tissue
10 React;20(1):3-15 (1998), which are all hereby incorporated by reference).

Polypeptides, including protein fusions to, or fragments thereof, of the present invention are useful in inhibiting the metastasis of proliferative cells or tissues. Inhibition may occur as a direct result of administering polypeptides, or antibodies directed to said polypeptides as described elsewhere herein, or indirectly, such as activating the expression of
15 proteins known to inhibit metastasis, for example alpha 4 integrins, (See, e.g., Curr Top Microbiol Immunol 1998;231:125-41, which is hereby incorporated by reference). Such therapeutic affects of the present invention may be achieved either alone, or in combination with small molecule drugs or adjuvants.

In another embodiment, the invention provides a method of delivering compositions
20 containing the polypeptides of the invention (e.g., compositions containing polypeptides or polypeptide antibodies associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs) to targeted cells expressing the polypeptide of the present invention. Polypeptides or polypeptide antibodies of the invention may be associated with with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic,
25 hydrophilic, ionic and/or covalent interactions.

Polypeptides, protein fusions to, or fragments thereof, of the present invention are useful in enhancing the immunogenicity and/or antigenicity of proliferating cells or tissues, either directly, such as would occur if the polypeptides of the present invention 'vaccinated' the immune response to respond to proliferative antigens and immunogens, or indirectly,
30 such as in activating the expression of proteins known to enhance the immune response (e.g. chemokines), to said antigens and immunogens.

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Cardiovascular Disorders

Polynucleotides or polypeptides, or agonists or antagonists of the present invention, may be used to treat cardiovascular disorders, including peripheral artery disease, such as limb ischemia.

5 Cardiovascular disorders include cardiovascular abnormalities, such as arterio-arterial fistula, arteriovenous fistula, cerebral arteriovenous malformations, congenital heart defects, pulmonary atresia, and Scimitar Syndrome. Congenital heart defects include aortic coarctation, cor triatriatum, coronary vessel anomalies, crisscross heart, dextrocardia, patent ductus arteriosus, Ebstein's anomaly, Eisenmenger complex, hypoplastic left heart syndrome, 10 levocardia, tetralogy of fallot, transposition of great vessels, double outlet right ventricle, tricuspid atresia, persistent truncus arteriosus, and heart septal defects, such as aortopulmonary septal defect, endocardial cushion defects, Lutembacher's Syndrome, trilogy of Fallot, ventricular heart septal defects.

 Cardiovascular disorders also include heart disease, such as arrhythmias, carcinoid 15 heart disease, high cardiac output, low cardiac output, cardiac tamponade, endocarditis (including bacterial), heart aneurysm, cardiac arrest, congestive heart failure, congestive cardiomyopathy, paroxysmal dyspnea, cardiac edema, heart hypertrophy, congestive cardiomyopathy, left ventricular hypertrophy, right ventricular hypertrophy, post-infarction heart rupture, ventricular septal rupture, heart valve diseases, myocardial diseases, 20 myocardial ischemia, pericardial effusion, pericarditis (including constrictive and tuberculous), pneumopericardium, postpericardiotomy syndrome, pulmonary heart disease, rheumatic heart disease, ventricular dysfunction, hyperemia, cardiovascular pregnancy complications, Scimitar Syndrome, cardiovascular syphilis, and cardiovascular tuberculosis.

 Arrhythmias include sinus arrhythmia, atrial fibrillation, atrial flutter, bradycardia, 25 extrasystole, Adams-Stokes Syndrome, bundle-branch block, sinoatrial block, long QT syndrome, parasystole, Lown-Ganong-Levine Syndrome, Mahaim-type pre-excitation syndrome, Wolff-Parkinson-White syndrome, sick sinus syndrome, tachycardias, and ventricular fibrillation. Tachycardias include paroxysmal tachycardia, supraventricular tachycardia, accelerated idioventricular rhythm, atrioventricular nodal reentry tachycardia, 30 ectopic atrial tachycardia, ectopic junctional tachycardia, sinoatrial nodal reentry tachycardia, sinus tachycardia, Torsades de Pointes, and ventricular tachycardia.

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Heart valve disease include aortic valve insufficiency, aortic valve stenosis, hear
murmurs, aortic valve prolapse, mitral valve prolapse, tricuspid valve prolapse, mitral valve
insufficiency, mitral valve stenosis, pulmonary atresia, pulmonary valve insufficiency,
pulmonary valve stenosis, tricuspid atresia, tricuspid valve insufficiency, and tricuspid valve
5 stenosis.

Myocardial diseases include alcoholic cardiomyopathy, congestive cardiomyopathy,
hypertrophic cardiomyopathy, aortic subvalvular stenosis, pulmonary subvalvular stenosis,
restrictive cardiomyopathy, Chagas cardiomyopathy, endocardial fibroelastosis,
endomyocardial fibrosis, Kearns Syndrome, myocardial reperfusion injury, and myocarditis.

10 Myocardial ischemias include coronary disease, such as angina pectoris, coronary
aneurysm, coronary arteriosclerosis, coronary thrombosis, coronary vasospasm, myocardial
infarction and myocardial stunning.

Cardiovascular diseases also include vascular diseases such as aneurysms,
angiodysplasia, angiomas, bacillary angiomas, Hippiel-Lindau Disease, Klippel-
15 Trenaunay-Weber Syndrome, Sturge-Weber Syndrome, angioneurotic edema, aortic diseases,
Takayasu's Arteritis, aortitis, Leriche's Syndrome, arterial occlusive diseases, arteritis,
enarteritis, polyarteritis nodosa, cerebrovascular disorders, diabetic angiopathies, diabetic
retinopathy, embolisms, thrombosis, erythromelalgia, hemorrhoids, hepatic veno-occlusive
disease, hypertension, hypotension, ischemia, peripheral vascular diseases, phlebitis,
20 pulmonary veno-occlusive disease, Raynaud's disease, CREST syndrome, retinal vein
occlusion, Scimitar syndrome, superior vena cava syndrome, telangiectasia, atacia
telangiectasia, hereditary hemorrhagic telangiectasia, varicocele, varicose veins, varicose
ulcer, vasculitis, and venous insufficiency.

Aneurysms include dissecting aneurysms, false aneurysms, infected aneurysms,
25 ruptured aneurysms, aortic aneurysms, cerebral aneurysms, coronary aneurysms, heart
aneurysms, and iliac aneurysms.

Arterial occlusive diseases include arteriosclerosis, intermittent claudication, carotid
stenosis, fibromuscular dysplasias, mesenteric vascular occlusion, Moyamoya disease, renal
artery obstruction, retinal artery occlusion, and thromboangiitis obliterans.

30 Cerebrovascular disorders include carotid artery diseases, cerebral amyloid
angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral
arteriovenous malformation, cerebral artery diseases, cerebral embolism and thrombosis,

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carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, cerebral hemorrhage, epidural hematoma, subdural hematoma, subarachnoid hemorrhage, cerebral infarction, cerebral ischemia (including transient), subclavian steal syndrome, periventricular leukomalacia, vascular headache, cluster headache, migraine, and vertebrobasilar
5 insufficiency.

Embolisms include air embolisms, amniotic fluid embolisms, cholesterol embolisms, blue toe syndrome, fat embolisms, pulmonary embolisms, and thromboembolisms. Thrombosis include coronary thrombosis, hepatic vein thrombosis, retinal vein occlusion, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, and thrombophlebitis.

10 Ischemia includes cerebral ischemia, ischemic colitis, compartment syndromes, anterior compartment syndrome, myocardial ischemia, reperfusion injuries, and peripheral limb ischemia. Vasculitis includes aortitis, arteritis, Behcet's Syndrome, Churg-Strauss Syndrome, mucocutaneous lymph node syndrome, thromboangiitis obliterans, hypersensitivity vasculitis, Schoenlein-Henoch purpura, allergic cutaneous vasculitis, and
15 Wegener's granulomatosis.

Polynucleotides or polypeptides, or agonists or antagonists of the present invention, are especially effective for the treatment of critical limb ischemia and coronary disease.

Polypeptides may be administered using any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical
20 administration, catheter infusion, biolistic injectors, particle accelerators, gelfoam sponge depots, other commercially available depot materials, osmotic pumps, oral or suppository solid pharmaceutical formulations, decanting or topical applications during surgery, aerosol delivery. Such methods are known in the art. Polypeptides may be administered as part of a Therapeutic, described in more detail below. Methods of delivering polynucleotides are
25 described in more detail herein.

Anti-Angiogenesis Activity

The naturally occurring balance between endogenous stimulators and inhibitors of angiogenesis is one in which inhibitory influences predominate. Rastinejad *et al.*, *Cell*
30 56:345-355 (1989). In those rare instances in which neovascularization occurs under normal physiological conditions, such as wound healing, organ regeneration, embryonic development, and female reproductive processes, angiogenesis is stringently regulated and

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spatially and temporally delimited. Under conditions of pathological angiogenesis such as that characterizing solid tumor growth, these regulatory controls fail. Unregulated angiogenesis becomes pathologic and sustains progression of many neoplastic and non-neoplastic diseases. A number of serious diseases are dominated by abnormal neovascularization including solid tumor growth and metastases, arthritis, some types of eye disorders, and psoriasis. See, e.g., reviews by Moses *et al.*, *Biotech.* 9:630-634 (1991); Folkman *et al.*, *N. Engl. J. Med.*, 333:1757-1763 (1995); Auerbach *et al.*, *J. Microvasc. Res.* 29:401-411 (1985); Folkman, *Advances in Cancer Research*, eds. Klein and Weinhouse, Academic Press, New York, pp. 175-203 (1985); Patz, *Am. J. Ophthalmol.* 94:715-743 (1982); and Folkman *et al.*, *Science* 221:719-725 (1983). In a number of pathological conditions, the process of angiogenesis contributes to the disease state. For example, significant data have accumulated which suggest that the growth of solid tumors is dependent on angiogenesis. Folkman and Klagsbrun, *Science* 235:442-447 (1987).

The present invention provides for treatment of diseases or disorders associated with neovascularization by administration of the polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists of the present invention. Malignant and metastatic conditions which can be treated with the polynucleotides and polypeptides, or agonists or antagonists of the invention include, but are not limited to, malignancies, solid tumors, and cancers described herein and otherwise known in the art (for a review of such disorders, see Fishman *et al.*, *Medicine*, 2d Ed., J. B. Lippincott Co., Philadelphia (1985)). Thus, the present invention provides a method of treating an angiogenesis-related disease and/or disorder, comprising administering to an individual in need thereof a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist of the invention. For example, polynucleotides, polypeptides, antagonists and/or agonists may be utilized in a variety of additional methods in order to therapeutically treat a cancer or tumor. Cancers which may be treated with polynucleotides, polypeptides, antagonists and/or agonists include, but are not limited to solid tumors, including prostate, lung, breast, ovarian, stomach, pancreas, larynx, esophagus, testes, liver, parotid, biliary tract, colon, rectum, cervix, uterus, endometrium, kidney, bladder, thyroid cancer; primary tumors and metastases; melanomas; glioblastoma; Kaposi's sarcoma; leiomyosarcoma; non-small cell lung cancer; colorectal cancer; advanced malignancies; and blood born tumors such as leukemias. For example, polynucleotides, polypeptides, antagonists and/or agonists may be delivered

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topically, in order to treat cancers such as skin cancer, head and neck tumors, breast tumors, and Kaposi's sarcoma.

Within yet other aspects, polynucleotides, polypeptides, antagonists and/or agonists may be utilized to treat superficial forms of bladder cancer by, for example, intravesical administration. Polynucleotides, polypeptides, antagonists and/or agonists may be delivered
5 directly into the tumor, or near the tumor site, via injection or a catheter. Of course, as the artisan of ordinary skill will appreciate, the appropriate mode of administration will vary according to the cancer to be treated. Other modes of delivery are discussed herein.

Polynucleotides, polypeptides, antagonists and/or agonists may be useful in treating
10 other disorders, besides cancers, which involve angiogenesis. These disorders include, but are not limited to: benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; arteriosclerotic plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis,
15 retinoblastoma, uveitis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque
20 neovascularization; telangiectasia; hemophilic joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis.

For example, within one aspect of the present invention methods are provided for treating hypertrophic scars and keloids, comprising the step of administering a polynucleotide, polypeptide, antagonist and/or agonist of the invention to a hypertrophic scar
25 or keloid.

Within one embodiment of the present invention polynucleotides, polypeptides, antagonists and/or agonists are directly injected into a hypertrophic scar or keloid, in order to prevent the progression of these lesions. This therapy is of particular value in the prophylactic treatment of conditions which are known to result in the development of
30 hypertrophic scars and keloids (e.g., burns), and is preferably initiated after the proliferative phase has had time to progress (approximately 14 days after the initial injury), but before hypertrophic scar or keloid development. As noted above, the present invention also

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provides methods for treating neovascular diseases of the eye, including for example, corneal neovascularization, neovascular glaucoma, proliferative diabetic retinopathy, retrolental fibroplasia and macular degeneration.

Moreover, Ocular disorders associated with neovascularization which can be treated
5 with the polynucleotides and polypeptides of the present invention (including agonists and/or antagonists) include, but are not limited to: neovascular glaucoma, diabetic retinopathy, retinoblastoma, retrolental fibroplasia, uveitis, retinopathy of prematurity macular degeneration, corneal graft neovascularization, as well as other eye inflammatory diseases, ocular tumors and diseases associated with choroidal or iris neovascularization. See, e.g.,
10 reviews by Waltman *et al.*, *Am. J. Ophthalmol.* 85:704-710 (1978) and Gartner *et al.*, *Surv. Ophthalmol.* 22:291-312 (1978).

Thus, within one aspect of the present invention methods are provided for treating neovascular diseases of the eye such as corneal neovascularization (including corneal graft neovascularization), comprising the step of administering to a patient a therapeutically
15 effective amount of a compound (as described above) to the cornea, such that the formation of blood vessels is inhibited. Briefly, the cornea is a tissue which normally lacks blood vessels. In certain pathological conditions however, capillaries may extend into the cornea from the pericorneal vascular plexus of the limbus. When the cornea becomes vascularized, it also becomes clouded, resulting in a decline in the patient's visual acuity. Visual loss may
20 become complete if the cornea completely opacitates. A wide variety of disorders can result in corneal neovascularization, including for example, corneal infections (e.g., trachoma, herpes simplex keratitis, leishmaniasis and onchocerciasis), immunological processes (e.g., graft rejection and Stevens-Johnson's syndrome), alkali burns, trauma, inflammation (of any cause), toxic and nutritional deficiency states, and as a complication of wearing contact
25 lenses.

Within particularly preferred embodiments of the invention, may be prepared for topical administration in saline (combined with any of the preservatives and antimicrobial agents commonly used in ocular preparations), and administered in eyedrop form. The solution or suspension may be prepared in its pure form and administered several times daily.
30 Alternatively, anti-angiogenic compositions, prepared as described above, may also be administered directly to the cornea. Within preferred embodiments, the anti-angiogenic composition is prepared with a muco-adhesive polymer which binds to cornea. Within

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further embodiments, the anti-angiogenic factors or anti-angiogenic compositions may be utilized as an adjunct to conventional steroid therapy. Topical therapy may also be useful prophylactically in corneal lesions which are known to have a high probability of inducing an angiogenic response (such as chemical burns). In these instances the treatment, likely in
5 combination with steroids, may be instituted immediately to help prevent subsequent complications.

Within other embodiments, the compounds described above may be injected directly into the corneal stroma by an ophthalmologist under microscopic guidance. The preferred site of injection may vary with the morphology of the individual lesion, but the goal of the
10 administration would be to place the composition at the advancing front of the vasculature (i.e., interspersed between the blood vessels and the normal cornea). In most cases this would involve perilimbal corneal injection to "protect" the cornea from the advancing blood vessels. This method may also be utilized shortly after a corneal insult in order to prophylactically prevent corneal neovascularization. In this situation the material could be
15 injected in the perilimbal cornea interspersed between the corneal lesion and its undesired potential limbal blood supply. Such methods may also be utilized in a similar fashion to prevent capillary invasion of transplanted corneas. In a sustained-release form injections might only be required 2-3 times per year. A steroid could also be added to the injection solution to reduce inflammation resulting from the injection itself.

20 Within another aspect of the present invention, methods are provided for treating neovascular glaucoma, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. In one embodiment, the compound may be administered topically to the eye in order to treat early forms of neovascular glaucoma.
25 Within other embodiments, the compound may be implanted by injection into the region of the anterior chamber angle. Within other embodiments, the compound may also be placed in any location such that the compound is continuously released into the aqueous humor. Within another aspect of the present invention, methods are provided for treating proliferative diabetic retinopathy, comprising the step of administering to a patient a
30 therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eyes, such that the formation of blood vessels is inhibited.

Within particularly preferred embodiments of the invention, proliferative diabetic

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retinopathy may be treated by injection into the aqueous humor or the vitreous, in order to increase the local concentration of the polynucleotide, polypeptide, antagonist and/or agonist in the retina. Preferably, this treatment should be initiated prior to the acquisition of severe disease requiring photocoagulation.

5 Within another aspect of the present invention, methods are provided for treating retrolental fibroplasia, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. The compound may be administered topically, via intravitreal injection and/or via intraocular implants.

10 Additionally, disorders which can be treated with the polynucleotides, polypeptides, agonists and/or antagonists include, but are not limited to, hemangioma, arthritis, psoriasis, angiofibroma, atherosclerotic plaques, delayed wound healing, granulations, hemophilic joints, hypertrophic scars, nonunion fractures, Osler-Weber syndrome, pyogenic granuloma, scleroderma, trachoma, and vascular adhesions.

15 Moreover, disorders and/or states, which can be treated with the the polynucleotides, polypeptides, agonists and/or antagonists include, but are not limited to, solid tumors, blood born tumors such as leukemias, tumor metastasis, Kaposi's sarcoma, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, rheumatoid arthritis, psoriasis, ocular angiogenic diseases, for
20 example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, and uveitis, delayed wound healing, endometriosis, vasculogenesis, granulations, hypertrophic scars (keloids), nonunion fractures, scleroderma, trachoma, vascular adhesions, myocardial angiogenesis, coronary collaterals, cerebral collaterals, arteriovenous malformations,
25 ischemic limb angiogenesis, Osler-Webber Syndrome, plaque neovascularization, telangiectasia, hemophilic joints, angiofibroma fibromuscular dysplasia, wound granulation, Crohn's disease, atherosclerosis, birth control agent by preventing vascularization required for embryo implantation controlling menstruation, diseases that have angiogenesis as a pathologic consequence such as cat scratch disease (*Rochela minalia quintosa*), ulcers
30 (*Helicobacter pylori*), Bartonellosis and bacillary angiomatosis.

 In one aspect of the birth control method, an amount of the compound sufficient to block embryo implantation is administered before or after intercourse and fertilization have

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occurred, thus providing an effective method of birth control, possibly a "morning after" method. Polynucleotides, polypeptides, agonists and/or agonists may also be used in controlling menstruation or administered as either a peritoneal lavage fluid or for peritoneal implantation in the treatment of endometriosis.

5 Polynucleotides, polypeptides, agonists and/or agonists of the present invention may be incorporated into surgical sutures in order to prevent stitch granulomas.

Polynucleotides, polypeptides, agonists and/or agonists may be utilized in a wide variety of surgical procedures. For example, within one aspect of the present invention a compositions (in the form of, for example, a spray or film) may be utilized to coat or spray an
10 area prior to removal of a tumor, in order to isolate normal surrounding tissues from malignant tissue, and/or to prevent the spread of disease to surrounding tissues. Within other aspects of the present invention, compositions (e.g., in the form of a spray) may be delivered via endoscopic procedures in order to coat tumors, or inhibit angiogenesis in a desired locale. Within yet other aspects of the present invention, surgical meshes which have been coated
15 with anti- angiogenic compositions of the present invention may be utilized in any procedure wherein a surgical mesh might be utilized. For example, within one embodiment of the invention a surgical mesh laden with an anti-angiogenic composition may be utilized during abdominal cancer resection surgery (e.g., subsequent to colon resection) in order to provide support to the structure, and to release an amount of the anti-angiogenic factor.

20 Within further aspects of the present invention, methods are provided for treating tumor excision sites, comprising administering a polynucleotide, polypeptide, agonist and/or agonist to the resection margins of a tumor subsequent to excision, such that the local recurrence of cancer and the formation of new blood vessels at the site is inhibited. Within one embodiment of the invention, the anti-angiogenic compound is administered directly to the
25 tumor excision site (e.g., applied by swabbing, brushing or otherwise coating the resection margins of the tumor with the anti-angiogenic compound). Alternatively, the anti-angiogenic compounds may be incorporated into known surgical pastes prior to administration. Within particularly preferred embodiments of the invention, the anti-angiogenic compounds are applied after hepatic resections for malignancy, and after neurosurgical operations.

30 Within one aspect of the present invention, polynucleotides, polypeptides, agonists and/or agonists may be administered to the resection margin of a wide variety of tumors, including for example, breast, colon, brain and hepatic tumors. For example, within one

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embodiment of the invention, anti-angiogenic compounds may be administered to the site of a neurological tumor subsequent to excision, such that the formation of new blood vessels at the site are inhibited.

The polynucleotides, polypeptides, agonists and/or antagonists of the present invention may also be administered along with other anti-angiogenic factors. Representative examples of other anti-angiogenic factors include: Anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel, Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include platelet factor 4; protamine

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5 sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, 1991); Sulphated Polysaccharide Peptidoglycan Complex (SP-PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including
10 for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem. 267:17321-17326, 1992); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, 1992); Cyclodextrin Tetradecasulfate; Eponemycin;
15 Camptothecin; Fumagillin (Ingber et al., Nature 348:555-557, 1990); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff, J. Clin. Invest. 79:1440-1446, 1987); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., J. Biol. Chem. 262(4):1659-1664, 1987); Bisantrone (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4-chloroanthronilic acid disodium or "CCA"; Takeuchi et al., Agents Actions 36:312-316,
20 1992); Thalidomide; Angostatic steroid; AGM-1470; carboxynaminolimidazole; and metalloproteinase inhibitors such as BB94.

Diseases at the Cellular Level

20 Diseases associated with increased cell survival or the inhibition of apoptosis that could be treated or detected by polynucleotides or polypeptides, as well as antagonists or agonists of the present invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma,
25 lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as
30 herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection. In preferred embodiments, polynucleotides,

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polypeptides, and/or antagonists of the invention are used to inhibit growth, progression, and/or metasis of cancers, in particular those listed above.

Additional diseases or conditions associated with increased cell survival that could be treated or detected by polynucleotides or polypeptides, or agonists or antagonists of the present invention include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma.

Diseases associated with increased apoptosis that could be treated or detected by polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, include AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host

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disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestasis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

5

Wound Healing and Epithelial Cell Proliferation

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, for therapeutic purposes, for example, to stimulate epithelial cell proliferation and basal keratinocytes for the purpose of wound healing, and to stimulate hair follicle production and healing of dermal wounds. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may be clinically useful in stimulating wound healing including surgical wounds, excisional wounds, deep wounds involving damage of the dermis and epidermis, eye tissue wounds, dental tissue wounds, oral cavity wounds, diabetic ulcers, dermal ulcers, cubitus ulcers, arterial ulcers, venous stasis ulcers, burns resulting from heat exposure or chemicals, and other abnormal wound healing conditions such as uremia, malnutrition, vitamin deficiencies and complications associated with systemic treatment with steroids, radiation therapy and antineoplastic drugs and antimetabolites. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to promote dermal reestablishment subsequent to dermal loss

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to increase the adherence of skin grafts to a wound bed and to stimulate re-epithelialization from the wound bed. The following are types of grafts that polynucleotides or polypeptides, agonists or antagonists of the present invention, could be used to increase adherence to a wound bed: autografts, artificial skin, allografts, autodermic graft, autoepdermic grafts, avacular grafts, Blair-Brown grafts, bone graft, brephoplastic grafts, cutis graft, delayed graft, dermic graft, epidermic graft, fascia graft, full thickness graft, heterologous graft, xenograft, homologous graft, hyperplastic graft, lamellar graft, mesh graft, mucosal graft, Ollier-Thiersch graft, omenpal graft, patch graft, pedicle graft, penetrating graft, split skin graft, thick split graft. Polynucleotides or polypeptides, as well as

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agonists or antagonists of the present invention, can be used to promote skin strength and to improve the appearance of aged skin.

It is believed that polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, will also produce changes in hepatocyte proliferation, and epithelial cell proliferation in the lung, breast, pancreas, stomach, small intestine, and large intestine. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could promote proliferation of epithelial cells such as sebocytes, hair follicles, hepatocytes, type II pneumocytes, mucin-producing goblet cells, and other epithelial cells and their progenitors contained within the skin, lung, liver, and gastrointestinal tract. Polynucleotides or polypeptides, agonists or antagonists of the present invention, may promote proliferation of endothelial cells, keratinocytes, and basal keratinocytes.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could also be used to reduce the side effects of gut toxicity that result from radiation, chemotherapy treatments or viral infections. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may have a cytoprotective effect on the small intestine mucosa. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may also stimulate healing of mucositis (mouth ulcers) that result from chemotherapy and viral infections.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could further be used in full regeneration of skin in full and partial thickness skin defects, including burns, (i.e., repopulation of hair follicles, sweat glands, and sebaceous glands), treatment of other skin defects such as psoriasis. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to treat epidermolysis bullosa, a defect in adherence of the epidermis to the underlying dermis which results in frequent, open and painful blisters by accelerating reepithelialization of these lesions. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could also be used to treat gastric and duodenal ulcers and help heal by scar formation of the mucosal lining and regeneration of glandular mucosa and duodenal mucosal lining more rapidly. Inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, are diseases which result in destruction of the mucosal surface of the small or large intestine, respectively. Thus, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to promote the resurfacing of the mucosal surface to aid

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more rapid healing and to prevent progression of inflammatory bowel disease. Treatment with polynucleotides or polypeptides, agonists or antagonists of the present invention, is expected to have a significant effect on the production of mucus throughout the gastrointestinal tract and could be used to protect the intestinal mucosa from injurious substances that are ingested or following surgery. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to treat diseases associated with the under expression.

Moreover, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to prevent and heal damage to the lungs due to various pathological states. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, which could stimulate proliferation and differentiation and promote the repair of alveoli and bronchiolar epithelium to prevent or treat acute or chronic lung damage. For example, emphysema, which results in the progressive loss of alveoli, and inhalation injuries, i.e., resulting from smoke inhalation and burns, that cause necrosis of the bronchiolar epithelium and alveoli could be effectively treated using polynucleotides or polypeptides, agonists or antagonists of the present invention. Also, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to stimulate the proliferation and differentiation of type II pneumocytes, which may help treat or prevent disease such as hyaline membrane diseases, such as infant respiratory distress syndrome and bronchopulmonary dysplasia, in premature infants.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could stimulate the proliferation and differentiation of hepatocytes and, thus, could be used to alleviate or treat liver diseases and pathologies such as fulminant liver failure caused by cirrhosis, liver damage caused by viral hepatitis and toxic substances (i.e., acetaminophen, carbon tetrachloride and other hepatotoxins known in the art).

In addition, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to treat or prevent the onset of diabetes mellitus. In patients with newly diagnosed Types I and II diabetes, where some islet cell function remains, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to maintain the islet function so as to alleviate, delay or prevent permanent manifestation of the disease. Also, polynucleotides or polypeptides, as well as agonists or

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antagonists of the present invention, could be used as an auxiliary in islet cell transplantation to improve or promote islet cell function.

Neural Activity and Neurological Diseases

5 The polynucleotides, polypeptides and agonists or antagonists of the invention may be used for the diagnosis and/or treatment of diseases, disorders, damage or injury of the brain and/or nervous system. Nervous system disorders that can be treated with the compositions of the invention (e.g., polypeptides, polynucleotides, and/or agonists or antagonists), include, but are not limited to, nervous system injuries, and diseases or disorders which result in either
10 a disconnection of axons, a diminution or degeneration of neurons, or demyelination. Nervous system lesions which may be treated in a patient (including human and non-human mammalian patients) according to the methods of the invention, include but are not limited to, the following lesions of either the central (including spinal cord, brain) or peripheral nervous systems: (1) ischemic lesions, in which a lack of oxygen in a portion of the nervous
15 system results in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia; (2) traumatic lesions, including lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the nervous system, or compression injuries; (3) malignant lesions, in which a portion of the nervous system is destroyed or injured by malignant tissue which is either a nervous system
20 associated malignancy or a malignancy derived from non-nervous system tissue; (4) infectious lesions, in which a portion of the nervous system is destroyed or injured as a result of infection, for example, by an abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, or syphilis; (5) degenerative lesions, in which a portion of the nervous system
25 is destroyed or injured as a result of a degenerative process including but not limited to, degeneration associated with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic lateral sclerosis (ALS); (6) lesions associated with nutritional diseases or disorders, in which a portion of the nervous system is destroyed or injured by a nutritional disorder or disorder of metabolism including, but not limited to, vitamin B12 deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia, Marchiafava-Bignami disease
30 (primary degeneration of the corpus callosum), and alcoholic cerebellar degeneration; (7) neurological lesions associated with systemic diseases including, but not limited to, diabetes

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(diabetic neuropathy, Bell's palsy), systemic lupus erythematosus, carcinoma, or sarcoidosis; (8) lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and (9) demyelinated lesions in which a portion of the nervous system is destroyed or injured by a demyelinating disease including, but not limited to, multiple sclerosis, human immunodeficiency virus-associated myelopathy, transverse myelopathy or various etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.

In one embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to protect neural cells from the damaging effects of hypoxia. In a further preferred embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to protect neural cells from the damaging effects of cerebral hypoxia. According to this embodiment, the compositions of the invention are used to treat or prevent neural cell injury associated with cerebral hypoxia. In one non-exclusive aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention, are used to treat or prevent neural cell injury associated with cerebral ischemia. In another non-exclusive aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat or prevent neural cell injury associated with cerebral infarction.

In another preferred embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat or prevent neural cell injury associated with a stroke. In a specific embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat or prevent cerebral neural cell injury associated with a stroke.

In another preferred embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat or prevent neural cell injury associated with a heart attack. In a specific embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat or prevent cerebral neural cell injury associated with a heart attack.

The compositions of the invention which are useful for treating or preventing a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons. For example, and not by way of limitation, compositions of the invention which elicit any of the following effects may be useful

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according to the invention: (1) increased survival time of neurons in culture either in the presence or absence of hypoxia or hypoxic conditions; (2) increased sprouting of neurons in culture or *in vivo*; (3) increased production of a neuron-associated molecule in culture or *in vivo*, e.g., choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or

5 (4) decreased symptoms of neuron dysfunction *in vivo*. Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may routinely be measured using a method set forth herein or otherwise known in the art, such as, for example, in Zhang *et al.*, *Proc Natl Acad Sci USA* 97:3637-42 (2000) or in Arakawa *et al.*, *J. Neurosci.*, 10:3507-15 (1990); increased sprouting of neurons may be

10 detected by methods known in the art, such as, for example, the methods set forth in Pestronk *et al.*, *Exp. Neurol.*, 70:65-82 (1980), or Brown *et al.*, *Ann. Rev. Neurosci.*, 4:17-42 (1981); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, etc., using techniques known in the art and depending on the molecule to be measured; and motor neuron dysfunction may be

15 measured by assessing the physical manifestation of motor neuron disorder, e.g., weakness, motor neuron conduction velocity, or functional disability.

In specific embodiments, motor neuron disorders that may be treated according to the invention include, but are not limited to, disorders such as infarction, infection, exposure to toxin, trauma, surgical damage, degenerative disease or malignancy that may affect motor

20 neurons as well as other components of the nervous system, as well as disorders that selectively affect neurons such as amyotrophic lateral sclerosis, and including, but not limited to, progressive spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary Motorsensory

25 Neuropathy (Charcot-Marie-Tooth Disease).

Further, polypeptides or polynucleotides of the invention may play a role in neuronal survival; synapse formation; conductance; neural differentiation, etc. Thus, compositions of the invention (including polynucleotides, polypeptides, and agonists or antagonists) may be used to diagnose and/or treat or prevent diseases or disorders associated with these roles,

30 including, but not limited to, learning and/or cognition disorders. The compositions of the invention may also be useful in the treatment or prevention of neurodegenerative disease states and/or behavioural disorders. Such neurodegenerative disease states and/or behavioral

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disorders include, but are not limited to, Alzheimers Disease, Parkinsons Disease, Huntingtons Disease, Tourette Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception.

5 In addition, compositions of the invention may also play a role in the treatment, prevention and/or detection of developmental disorders associated with the developing embryo, or sexually-linked disorders.

Additionally, polypeptides, polynucleotides and/or agonists or antagonists of the invention, may be useful in protecting neural cells from diseases, damage, disorders, or injury, associated with cerebrovascular disorders including, but not limited to, carotid artery diseases (e.g., carotid artery thrombosis, carotid stenosis, or Moyamoya Disease), cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformations, cerebral artery diseases, cerebral embolism and thrombosis (e.g., carotid artery thrombosis, sinus thrombosis, or Wallenberg's Syndrome), cerebral hemorrhage (e.g., epidural or subdural hematoma, or subarachnoid hemorrhage), cerebral infarction, cerebral ischemia (e.g., transient cerebral ischemia, Subclavian Steal Syndrome, or vertebrobasilar insufficiency), vascular dementia (e.g., multi-infarct), leukomalacia, periventricular, and vascular headache (e.g., cluster headache or migraines).

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, for therapeutic purposes, for example, to stimulate neurological cell proliferation and/or differentiation. Therefore, polynucleotides, polypeptides, agonists and/or antagonists of the invention may be used to treat and/or detect neurologic diseases. Moreover, polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used as a marker or detector of a particular nervous system disease or disorder.

Examples of neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include brain diseases, such as metabolic brain diseases which includes phenylketonuria such as maternal phenylketonuria, pyruvate carboxylase deficiency, pyruvate dehydrogenase complex deficiency, Wernicke's Encephalopathy, brain edema, brain neoplasms such as cerebellar neoplasms which include infratentorial neoplasms, cerebral ventricle neoplasms such as choroid plexus neoplasms, hypothalamic neoplasms, supratentorial neoplasms,

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canavan disease, cerebellar diseases such as cerebellar ataxia which include spinocerebellar degeneration such as ataxia telangiectasia, cerebellar dyssynergia, Friederich's Ataxia, Machado-Joseph Disease, olivopontocerebellar atrophy, cerebellar neoplasms such as infratentorial neoplasms, diffuse cerebral sclerosis such as encephalitis periaxialis, globoid cell leukodystrophy, metachromatic leukodystrophy and subacute sclerosing panencephalitis.

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include cerebrovascular disorders (such as carotid artery diseases which include carotid artery thrombosis, carotid stenosis and Moyamoya Disease), cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformations, cerebral artery diseases, cerebral embolism and thrombosis such as carotid artery thrombosis, sinus thrombosis and Wallenberg's Syndrome, cerebral hemorrhage such as epidural hematoma, subdural hematoma and subarachnoid hemorrhage, cerebral infarction, cerebral ischemia such as transient cerebral ischemia, Subclavian Steal Syndrome and vertebrobasilar insufficiency, vascular dementia such as multi-infarct dementia, periventricular leukomalacia, vascular headache such as cluster headache and migraine.

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include dementia such as AIDS Dementia Complex, presenile dementia such as Alzheimer's Disease and Creutzfeldt-Jakob Syndrome, senile dementia such as Alzheimer's Disease and progressive supranuclear palsy, vascular dementia such as multi-infarct dementia, encephalitis which include encephalitis periaxialis, viral encephalitis such as epidemic encephalitis, Japanese Encephalitis, St. Louis Encephalitis, tick-borne encephalitis and West Nile Fever, acute disseminated encephalomyelitis, meningoencephalitis such as uveomeningoencephalitic syndrome, Postencephalitic Parkinson Disease and subacute sclerosing panencephalitis, encephalomalacia such as periventricular leukomalacia, epilepsy such as generalized epilepsy which includes infantile spasms, absence epilepsy, myoclonic epilepsy which includes MERRF Syndrome, tonic-clonic epilepsy, partial epilepsy such as complex partial epilepsy, frontal lobe epilepsy and temporal lobe epilepsy, post-traumatic epilepsy, status epilepticus such as Epilepsia Partialis Continua, and Hallervorden-Spatz Syndrome.

2015

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include hydrocephalus such as Dandy-Walker Syndrome and normal pressure hydrocephalus, hypothalamic diseases such as hypothalamic neoplasms, cerebral malaria, narcolepsy which
5 includes cataplexy, bulbar poliomyelitis, cerebri pseudotumor, Rett Syndrome, Reye's Syndrome, thalamic diseases, cerebral toxoplasmosis, intracranial tuberculoma and Zellweger Syndrome, central nervous system infections such as AIDS Dementia Complex, Brain Abscess, subdural empyema, encephalomyelitis such as Equine Encephalomyelitis, Venezuelan Equine Encephalomyelitis, Necrotizing Hemorrhagic Encephalomyelitis, Visna,
10 and cerebral malaria.

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include meningitis such as arachnoiditis, aseptic meningitis such as viral meningitis which includes lymphocytic choriomeningitis, Bacterial meningitis which includes Haemophilus Meningitis, Listeria
15 Meningitis, Meningococcal Meningitis such as Waterhouse-Friderichsen Syndrome, Pneumococcal Meningitis and meningeal tuberculosis, fungal meningitis such as Cryptococcal Meningitis, subdural effusion, meningoencephalitis such as uvemeningoencephalitic syndrome, myelitis such as transverse myelitis, neurosyphilis such as tabes dorsalis, poliomyelitis which includes bulbar poliomyelitis and postpoliomyelitis
20 syndrome, prion diseases (such as Creutzfeldt-Jakob Syndrome, Bovine Spongiform Encephalopathy, Gerstmann-Straussler Syndrome, Kuru, Scrapie), and cerebral toxoplasmosis.

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include central nervous
25 system neoplasms such as brain neoplasms that include cerebellar neoplasms such as infratentorial neoplasms, cerebral ventricle neoplasms such as choroid plexus neoplasms, hypothalamic neoplasms and supratentorial neoplasms, meningeal neoplasms, spinal cord neoplasms which include epidural neoplasms, demyelinating diseases such as Canavan Diseases, diffuse cerebral scleritis which includes adrenoleukodystrophy, encephalitis periaxialis, globoid cell leukodystrophy, diffuse cerebral sclerosis such as metachromatic leukodystrophy, allergic encephalomyelitis, necrotizing hemorrhagic encephalomyelitis,
30 progressive multifocal leukoencephalopathy, multiple sclerosis, central pontine myelinolysis,

2016

transverse myelitis, neuromyelitis optica, Scrapie, Swayback, Chronic Fatigue Syndrome, Visna, High Pressure Nervous Syndrome, Meningism, spinal cord diseases such as amyotonia congenita, amyotrophic lateral sclerosis, spinal muscular atrophy such as Werdnig-Hoffmann Disease, spinal cord compression, spinal cord neoplasms such as epidural neoplasms, syringomyelia, Tabes Dorsalis, Stiff-Man Syndrome, mental retardation such as Angelman Syndrome, Cri-du-Chat Syndrome, De Lange's Syndrome, Down Syndrome, Gangliosidoses such as gangliosidoses G(M1), Sandhoff Disease, Tay-Sachs Disease, Hartnup Disease, homocystinuria, Laurence-Moon- Biedl Syndrome, Lesch-Nyhan Syndrome, Maple Syrup Urine Disease, mucopolysaccharidosis such as fucosidosis, neuronal ceroid-lipofuscinosis, oculocerebrorenal syndrome, phenylketonuria such as maternal phenylketonuria, Prader-Willi Syndrome, Rett Syndrome, Rubinstein-Taybi Syndrome, Tuberous Sclerosis, WAGR Syndrome, nervous system abnormalities such as holoprosencephaly, neural tube defects such as anencephaly which includes hydranencephaly, Arnold-Chiari Deformity, encephalocele, meningocele, meningomyelocele, spinal dysraphism such as spina bifida cystica and spina bifida occulta.

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include hereditary motor and sensory neuropathies which include Charcot-Marie Disease, Hereditary optic atrophy, Refsum's Disease, hereditary spastic paraplegia, Werdnig-Hoffmann Disease, Hereditary Sensory and Autonomic Neuropathies such as Congenital Analgesia and Familial Dysautonomia, Neurologic manifestations (such as agnosia that include Gerstmann's Syndrome, Amnesia such as retrograde amnesia, apraxia, neurogenic bladder, cataplexy, communicative disorders such as hearing disorders that includes deafness, partial hearing loss, loudness recruitment and tinnitus, language disorders such as aphasia which include agraphia, anomia, Broca's aphasia, and Wernicke's Aphasia, Dyslexia such as Acquired Dyslexia, language development disorders, speech disorders such as aphasia which includes anomia, Broca's aphasia and Wernicke's Aphasia, articulation disorders, communicative disorders such as speech disorders which include dysarthria, echolalia, mutism and stuttering, voice disorders such as aphonia and hoarseness, decerebrate state, delirium, fasciculation, hallucinations, meningism, movement disorders such as Angelman syndrome, ataxia, athetosis, chorea, dystonia, hypokinesia, muscle hypotonia, myoclonus, tic, torticollis and tremor, muscle hypertonia such as muscle rigidity such as stiff-man syndrome, muscle

2017

spasticity, paralysis such as facial paralysis which includes Herpes Zoster Oticus, Gastroparesis, Hemiplegia, ophthalmoplegia such as diplopia, Duane's Syndrome, Horner's Syndrome, Chronic progressive external ophthalmoplegia such as Kearns Syndrome, Bulbar Paralysis, Tropical Spastic Paraparesis, Paraplegia such as Brown-Sequard Syndrome, 5 quadriplegia, respiratory paralysis and vocal cord paralysis, paresis, phantom limb, taste disorders such as ageusia and dysgeusia, vision disorders such as amblyopia, blindness, color vision defects, diplopia, hemianopsia, scotoma and subnormal vision, sleep disorders such as hypersomnia which includes Kleine-Levin Syndrome, insomnia, and somnambulism, spasm such as trismus, unconsciousness such as coma, persistent vegetative state and syncope and 10 vertigo, neuromuscular diseases such as amyotonia congenita, amyotrophic lateral sclerosis, Lambert-Eaton Myasthenic Syndrome, motor neuron disease, muscular atrophy such as spinal muscular atrophy, Charcot-Marie Disease and Werdnig-Hoffmann Disease, Postpoliomyelitis Syndrome, Muscular Dystrophy, Myasthenia Gravis, Myotonia Atrophica, Myotonia Confenita, Nemaline Myopathy, Familial Periodic Paralysis, Multiplex 15 Paramyoclonus, Tropical Spastic Paraparesis and Stiff-Man Syndrome, peripheral nervous system diseases such as acrodynia, amyloid neuropathies, autonomic nervous system diseases such as Adie's Syndrome, Barre-Lieou Syndrome, Familial Dysautonomia, Horner's Syndrome, Reflex Sympathetic Dystrophy and Shy-Drager Syndrome, Cranial Nerve Diseases such as Acoustic Nerve Diseases such as Acoustic Neuroma which includes 20 Neurofibromatosis 2, Facial Nerve Diseases such as Facial Neuralgia, Melkersson-Rosenthal Syndrome, ocular motility disorders which includes amblyopia, nystagmus, oculomotor nerve paralysis, ophthalmoplegia such as Duane's Syndrome, Horner's Syndrome, Chronic Progressive External Ophthalmoplegia which includes Kearns Syndrome, Strabismus such as Esotropia and Exotropia, Oculomotor Nerve Paralysis, Optic Nerve Diseases such as Optic 25 Atrophy which includes Hereditary Optic Atrophy, Optic Disk Drusen, Optic Neuritis such as Neuromyelitis Optica, Papilledema, Trigeminal Neuralgia, Vocal Cord Paralysis, Demyelinating Diseases such as Neuromyelitis Optica and Swayback, and Diabetic neuropathies such as diabetic foot.

Additional neurologic diseases which can be treated or detected with polynucleotides, 30 polypeptides, agonists, and/or antagonists of the present invention include nerve compression syndromes such as carpal tunnel syndrome, tarsal tunnel syndrome, thoracic outlet syndrome such as cervical rib syndrome, ulnar nerve compression syndrome, neuralgia such as

2018

causalgia, cervico-brachial neuralgia, facial neuralgia and trigeminal neuralgia, neuritis such as experimental allergic neuritis, optic neuritis, polyneuritis, polyradiculoneuritis and radiculities such as polyradiculitis, hereditary motor and sensory neuropathies such as Charcot-Marie Disease, Hereditary Optic Atrophy, Refsum's Disease, Hereditary Spastic
5 Paraplegia and Werdnig-Hoffmann Disease, Hereditary Sensory and Autonomic Neuropathies which include Congenital Analgesia and Familial Dysautonomia, POEMS Syndrome, Sciatica, Gustatory Sweating and Tetany).

10 **Infectious Disease**

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention can be used to treat or detect infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated. The immune response may be increased by either
15 enhancing an existing immune response, or by initiating a new immune response. Alternatively, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

Viruses are one example of an infectious agent that can cause disease or symptoms
20 that can be treated or detected by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention. Examples of viruses, include, but are not limited to Examples of viruses, include, but are not limited to the following DNA and RNA viruses and viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Bimaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Dengue, EBV, HIV, Flaviviridae, Hepadnaviridae
25 (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza A, Influenza B, and parainfluenza), Papiloma virus, Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus).
30 Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiolitis, respiratory syncytial virus, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E,

2019

Chronic Active, Delta), Japanese B encephalitis, Junin, Chikungunya, Rift Valley fever, yellow fever, meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used to treat or detect any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat: meningitis, Dengue, EBV, and/or hepatitis (e.g., hepatitis B). In an additional specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat patients nonresponsive to one or more other commercially available hepatitis vaccines. In a further specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat AIDS.

Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated or detected by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, include, but not limited to, the following Gram-Negative and Gram-positive bacteria and bacterial families and fungi: Actinomycetales (e.g., Corynebacterium, Mycobacterium, Norcardia), Cryptococcus neoformans, Aspergillosis, Bacillaceae (e.g., Anthrax, Clostridium), Bacteroidaceae, Blastomycosis, Bordetella, Borrelia (e.g., Borrelia burgdorferi, Brucellosis, Candidiasis, Campylobacter, Coccidioidomycosis, Cryptococcosis, Dermatocycoses, E. coli (e.g., Enterotoxigenic E. coli and Enterohemorrhagic E. coli), Enterobacteriaceae (Klebsiella, Salmonella (e.g., Salmonella typhi, and Salmonella paratyphi), Serratia, Yersinia), Erysipelothrix, Helicobacter, Legionellosis, Leptospirosis, Listeria, Mycoplasmatales, Mycobacterium leprae, Vibrio cholerae, Neisseriaceae (e.g., Acinetobacter, Gonorrhea, Meningococcal), Meisseria meningitidis, Pasteurellacea Infections (e.g., Actinobacillus, Heamophilus (e.g., Heamophilus influenza type B), Pasteurella), Pseudomonas, Rickettsiaceae, Chlamydiaceae, Syphilis, Shigella spp., Staphylococcal, Meningiococcal, Pneumococcal and Streptococcal (e.g., Streptococcus pneumoniae and Group B Streptococcus). These bacterial or fungal families can cause the following diseases or symptoms, including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme

2020

Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning, Typhoid, pneumonia, Gonorrhea, meningitis (e.g., meningitis types A and B), Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g.,
5 cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections. Polynucleotides or polypeptides, agonists or antagonists of the invention, can be used to treat or detect any of these symptoms or diseases. In specific embodiments, Polynucleotides, polypeptides, agonists or antagonists of the invention are used to treat: tetanus, Diphtheria, botulism, and/or meningitis type B.

10 Moreover, parasitic agents causing disease or symptoms that can be treated or detected by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, the following families or class: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and
15 Trichomonas and Sporozoans (e.g., Plasmodium virax, Plasmodium falciparum, Plasmodium malariae and Plasmodium ovale). These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), malaria, pregnancy complications, and toxoplasmosis.
20 polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used to treat or detect any of these symptoms or diseases.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention of the present invention could either be by administering an effective amount of a polypeptide to the patient, or by removing cells from the patient, supplying the cells with a
25 polynucleotide of the present invention, and returning the engineered cells to the patient (ex vivo therapy). Moreover, the polypeptide or polynucleotide of the present invention can be used as an antigen in a vaccine to raise an immune response against infectious disease.

Regeneration

30 Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues. (See, Science 276:59-87 (1997).) The regeneration of tissues could be used to

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repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteoarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

5 Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vasculature (including vascular and lymphatics), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

10 Moreover, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could
15 be treated include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

 Similarly, nerve and brain tissue could also be regenerated by using polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, to proliferate and
20 differentiate nerve cells. Diseases that could be treated using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic disorders (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stroke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system
25 diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated using the polynucleotides or polypeptides, as well as agonists or antagonists of the present invention.

Chemotaxis

30 Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may have chemotaxis activity. A chemotactic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or

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endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may increase chemotactic activity of particular cells. These chemotactic molecules can then be used to treat inflammation, infection, hyperproliferative disorders, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotactic molecules can be used to treat wounds and other trauma to tissues by attracting immune cells to the injured location. Chemotactic molecules of the present invention can also attract fibroblasts, which can be used to treat wounds.

It is also contemplated that polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may inhibit chemotactic activity. These molecules could also be used to treat disorders. Thus, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention could be used as an inhibitor of chemotaxis.

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Binding Activity

A polypeptide of the present invention may be used to screen for molecules that bind to the polypeptide or for molecules to which the polypeptide binds. The binding of the polypeptide and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of the polypeptide or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

Preferably, the molecule is closely related to the natural ligand of the polypeptide, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic. (See, Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991).) Similarly, the molecule can be closely related to the natural receptor to which the polypeptide binds, or at least, a fragment of the receptor capable of being bound by the polypeptide (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

Preferably, the screening for these molecules involves producing appropriate cells which express the polypeptide. Preferred cells include cells from mammals, yeast, *Drosophila*, or *E. coli*. Cells expressing the polypeptide (or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially

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containing the molecule to observe binding, stimulation, or inhibition of activity of either the polypeptide or the molecule.

The assay may simply test binding of a candidate compound to the polypeptide, wherein binding is detected by a label, or in an assay involving competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to the polypeptide.

Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide, measuring polypeptide/molecule activity or binding, and comparing the polypeptide/molecule activity or binding to a standard.

Preferably, an ELISA assay can measure polypeptide level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can measure polypeptide level or activity by either binding, directly or indirectly, to the polypeptide or by competing with the polypeptide for a substrate.

Additionally, the receptor to which the polypeptide of the present invention binds can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting (Coligan, et al., Current Protocols in Immun., 1(2), Chapter 5, (1991)). For example, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the polypeptides, for example, NIH3T3 cells which are known to contain multiple receptors for the FGF family proteins, and SC-3 cells, and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the polypeptides. Transfected cells which are grown on glass slides are exposed to the polypeptide of the present invention, after they have been labelled. The polypeptides can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase.

Following fixation and incubation, the slides are subjected to auto-radiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an iterative sub-pooling and re-screening process, eventually yielding a single clones that encodes the putative receptor.

As an alternative approach for receptor identification, the labeled polypeptides can be photoaffinity linked with cell membrane or extract preparations that express the receptor

molecule. Cross-linked material is resolved by PAGE analysis and exposed to X-ray film. The labeled complex containing the receptors of the polypeptides can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the genes encoding the putative receptors.

Moreover, the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling") may be employed to modulate the activities of the polypeptide of the present invention thereby effectively generating agonists and antagonists of the polypeptide of the present invention. *See generally*, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458, and Patten, P. A., *et al.*, *Curr. Opinion Biotechnol.* 8:724-33 (1997); Harayama, S. *Trends Biotechnol.* 16(2):76-82 (1998); Hansson, L. O., *et al.*, *J. Mol. Biol.* 287:265-76 (1999); and Lorenzo, M. M. and Blasco, R. *Biotechniques* 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference). In one embodiment, alteration of polynucleotides and corresponding polypeptides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments into a desired molecule by homologous, or site-specific, recombination. In another embodiment, polynucleotides and corresponding polypeptides may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of the polypeptide of the present invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules. In preferred embodiments, the heterologous molecules are family members. In further preferred embodiments, the heterologous molecule is a growth factor such as, for example, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-I), transforming growth factor (TGF)-alpha, epidermal growth factor (EGF), fibroblast growth factor (FGF), TGF-beta, bone morphogenetic protein (BMP)-2, BMP-4, BMP-5, BMP-6, BMP-7, activins A and B, decapentaplegic(dpp), 60A, OP-2, dorsalin, growth differentiation factors (GDFs), nodal, MIS, inhibin-alpha, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta5, and glial-derived neurotrophic factor (GDNF).

Other preferred fragments are biologically active fragments of the polypeptide of the present invention. Biologically active fragments are those exhibiting activity similar, but not

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necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

Additionally, this invention provides a method of screening compounds to identify
5 those which modulate the action of the polypeptide of the present invention. An example of such an assay comprises combining a mammalian fibroblast cell, a the polypeptide of the present invention, the compound to be screened and 3[H] thymidine under cell culture conditions where the fibroblast cell would normally proliferate. A control assay may be performed in the absence of the compound to be screened and compared to the amount of
10 fibroblast proliferation in the presence of the compound to determine if the compound stimulates proliferation by determining the uptake of 3[H] thymidine in each case. The amount of fibroblast cell proliferation is measured by liquid scintillation chromatography which measures the incorporation of 3[H] thymidine. Both agonist and antagonist compounds may be identified by this procedure.

15 In another method, a mammalian cell or membrane preparation expressing a receptor for a polypeptide of the present invention is incubated with a labeled polypeptide of the present invention in the presence of the compound. The ability of the compound to enhance or block this interaction could then be measured. Alternatively, the response of a known second messenger system following interaction of a compound to be screened and the
20 receptor is measured and the ability of the compound to bind to the receptor and elicit a second messenger response is measured to determine if the compound is a potential agonist or antagonist. Such second messenger systems include but are not limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis.

All of these above assays can be used as diagnostic or prognostic markers. The
25 molecules discovered using these assays can be used to treat disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the polypeptide/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of the polypeptides of the invention from suitably manipulated cells or tissues.

30 Therefore, the invention includes a method of identifying compounds which bind to a polypeptide of the invention comprising the steps of: (a) incubating a candidate binding compound with a colon and/or colon cancer polynucleotides and/or polypeptides polypeptide

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of the invention; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with a colon and/or colon cancer polynucleotides and/or polypeptides polypeptide of the invention, (b) assaying a biological activity, and (b) determining if a biological activity of the polypeptide has been altered.

Colon Cancer Antigen Binding Peptides and Other Molecules

The invention also encompasses screening methods for identifying polypeptides and nonpolypeptides that bind the colon cancer antigens of the invention, and the colon cancer antigen binding molecules identified thereby. These binding molecules are useful, for example, as agonists and antagonists of the colon cancer antigens of the invention. Such agonists and antagonists can be used, in accordance with the invention, in the therapeutic embodiments described in detail, below.

This method comprises the steps of:

- a. contacting a colon cancer antigen of the invention with a plurality of molecules; and
- b. identifying a molecule that binds the colon cancer antigen.

The step of contacting the colon cancer antigen of the invention with the plurality of molecules may be effected in a number of ways. For example, one may contemplate immobilizing the colon cancer antigen on a solid support and bringing a solution of the plurality of molecules in contact with the immobilized colon cancer antigen. Such a procedure would be akin to an affinity chromatographic process, with the affinity matrix being comprised of the immobilized colon cancer antigen of the invention. The molecules having a selective affinity for the colon cancer antigen can then be purified by affinity selection. The nature of the solid support, process for attachment of the colon cancer antigen of the invention to the solid support, solvent, and conditions of the affinity isolation or selection are largely conventional and well known to those of ordinary skill in the art.

Alternatively, one may also separate a plurality of polypeptides into substantially separate fractions comprising a subset of or individual polypeptides. For instance, one can separate the plurality of polypeptides by gel electrophoresis, column chromatography, or like method known to those of ordinary skill for the separation of polypeptides. The individual polypeptides can also be produced by a transformed host cell in such a way as to be

expressed on or about its outer surface (e.g., a recombinant phage). Individual isolates can then be "probed" by a colon cancer antigen, optionally in the presence of an inducer should one be required for expression, to determine if any selective affinity interaction takes place between the colon cancer antigen and the individual clone. Prior to contacting the colon cancer antigen of the invention with each fraction comprising individual polypeptides, the polypeptides could first be transferred to a solid support for additional convenience. Such a solid support may simply be a piece of filter membrane, such as one made of nitrocellulose or nylon. In this manner, positive clones could be identified from a collection of transformed host cells of an expression library, which harbor a DNA construct encoding a polypeptide having a selective affinity for protein of the invention. Furthermore, the amino acid sequence of the polypeptide having a selective affinity for a colon cancer antigen of the invention can be determined directly by conventional means or the coding sequence of the DNA encoding the polypeptide can frequently be determined more conveniently. The primary sequence can then be deduced from the corresponding DNA sequence. If the amino acid sequence is to be determined from the polypeptide itself, one may use microsequencing techniques. The sequencing technique may include mass spectroscopy.

In certain situations, it may be desirable to wash away any unbound colon cancer antigen, or alternatively, unbound polypeptides, from a mixture of the colon cancer antigen of the invention and the plurality of polypeptides prior to attempting to determine or to detect the presence of a selective affinity interaction. Such a wash step may be particularly desirable when the protein of the invention or the plurality of polypeptides is bound to a solid support.

The plurality of molecules provided according to this method may be provided by way of diversity libraries, such as random or combinatorial peptide or nonpeptide libraries which can be screened for molecules that specifically bind to a protein of the invention. Many libraries are known in the art that can be used, e.g., chemically synthesized libraries, recombinant (e.g., phage display libraries), and in vitro translation-based libraries. Examples of chemically synthesized libraries are described in Fodor et al., 1991, *Science* 251:767-773; Houghten et al., 1991, *Nature* 354:84-86; Lam et al., 1991, *Nature* 354:82-84; Medynski, 1994, *Bio/Technology* 12:709-710; Gallop et al., 1994, *J. Medicinal Chemistry* 37(9):1233-1251; Ohlmeyer et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:10922-10926; Erb et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:11422-11426; Houghten et al., 1992, *Biotechniques* 13:412; Jayawickreme et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:1614-1618; Salmon et al., 1993,

Proc. Natl. Acad. Sci. USA 90:11708-11712; PCT Publication No. WO 93/20242; and Brenner and Lerner, 1992, Proc. Natl. Acad. Sci. USA 89:5381-5383.

Examples of phage display libraries are described in Scott and Smith, 1990, Science 249:386-390; Devlin et al., 1990, Science, 249:404-406; Christian, R. B., et al., 1992, J. Mol. Biol. 227:711-718); Lenstra, 1992, J. Immunol. Meth. 152:149-157; Kay et al., 1993, Gene 128:59-65; and PCT Publication No. WO 94/18318 dated Aug. 18, 1994.

In vitro translation-based libraries include, but are not limited to, those described in PCT Publication No. WO 91/05058 dated Apr. 18, 1991; and Mattheakis et al., 1994, Proc. Natl. Acad. Sci. USA 91:9022-9026.

By way of examples of nonpeptide libraries, a benzodiazepine library (see e.g., Bunin et al., 1994, Proc. Natl. Acad. Sci. USA 91:4708-4712) can be adapted for use. Peptoid libraries (Simon et al., 1992, Proc. Natl. Acad. Sci. USA 89:9367-9371) can also be used. Another example of a library that can be used, in which the amide functionalities in peptides have been permethylated to generate a chemically transformed combinatorial library, is described by Ostresh et al. (1994, Proc. Natl. Acad. Sci. USA 91:11138-11142).

The variety of non-peptide libraries that are useful in the present invention is great. For example, Ecker and Crooke, 1995, Bio/Technology 13:351-360 list benzodiazepines, hydantoins, piperazinediones, biphenyls, sugar analogs, beta-mercaptoketones, arylacetic acids, acylpiperidines, benzopyrans, cubanes, xanthines, aminimides, and oxazolones as among the chemical species that form the basis of various libraries.

Non-peptide libraries can be classified broadly into two types: decorated monomers and oligomers. Decorated monomer libraries employ a relatively simple scaffold structure upon which a variety functional groups is added. Often the scaffold will be a molecule with a known useful pharmacological activity. For example, the scaffold might be the benzodiazepine structure.

Non-peptide oligomer libraries utilize a large number of monomers that are assembled together in ways that create new shapes that depend on the order of the monomers. Among the monomer units that have been used are carbamates, pyrrolinones, and morpholinos. Peptoids, peptide-like oligomers in which the side chain is attached to the alpha amino group rather than the alpha carbon, form the basis of another version of non-peptide oligomer libraries. The first non-peptide oligomer libraries utilized a single type of monomer and thus contained a repeating backbone. Recent libraries have utilized more than one

monomer, giving the libraries added flexibility.

Screening the libraries can be accomplished by any of a variety of commonly known methods. See, e.g., the following references, which disclose screening of peptide libraries: Parmley and Smith, 1989, *Adv. Exp. Med. Biol.* 251:215-218; Scott and Smith, 1990, *Science* 249:386-390; Fowlkes et al., 1992, *BioTechniques* 13:422-427; Oldenburg et al., 1992, *Proc. Natl. Acad. Sci. USA* 89:5393-5397; Yu et al., 1994, *Cell* 76:933-945; Staudt et al., 1988, *Science* 241:577-580; Bock et al., 1992, *Nature* 355:564-566; Tuerk et al., 1992, *Proc. Natl. Acad. Sci. USA* 89:6988-6992; Ellington et al., 1992, *Nature* 355:850-852; U.S. Pat. No. 5,096,815, U.S. Pat. No. 5,223,409, and U.S. Pat. No. 5,198,346, all to Ladner et al.;
10 Rebar and Pabo, 1993, *Science* 263:671-673; and CT Publication No. WO 94/18318.

In a specific embodiment, screening to identify a molecule that binds a colon cancer antigen can be carried out by contacting the library members with a colon cancer antigen of the invention immobilized on a solid phase and harvesting those library members that bind to the colon cancer antigen. Examples of such screening methods, termed "panning" techniques
15 are described by way of example in Parmley and Smith, 1988, *Gene* 73:305-318; Fowlkes et al., 1992, *BioTechniques* 13:422-427; PCT Publication No. WO 94/18318; and in references cited herein.

In another embodiment, the two-hybrid system for selecting interacting proteins in yeast (Fields and Song, 1989, *Nature* 340:245-246; Chien et al., 1991, *Proc. Natl. Acad. Sci.*
20 *USA* 88:9578-9582) can be used to identify molecules that specifically bind to a colon and/or colon cancer related protein of the invention.

Where a colon cancer antigen of the invention binding molecule is a polypeptide, the polypeptide can be conveniently selected from any peptide library, including random peptide libraries, combinatorial peptide libraries, or biased peptide libraries. The term "biased" is
25 used herein to mean that the method of generating the library is manipulated so as to restrict one or more parameters that govern the diversity of the resulting collection of molecules, in this case peptides.

Thus, a truly random peptide library would generate a collection of peptides in which the probability of finding a particular amino acid at a given position of the peptide is the same for all 20 amino acids. A bias can be introduced into the library, however, by
30 specifying, for example, that a lysine occur every fifth amino acid or that positions 4, 8, and 9 of a decapeptide library be fixed to include only arginine. Clearly, many types of biases can

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be contemplated, and the present invention is not restricted to any particular bias. Furthermore, the present invention contemplates specific types of peptide libraries, such as phage displayed peptide libraries and those that utilize a DNA construct comprising a lambda phage vector with a DNA insert.

5 As mentioned above, in the case of a colon and/or colon cancer related protein of the invention binding molecule that is a polypeptide, the polypeptide may have about 6 to less than about 60 amino acid residues, preferably about 6 to about 10 amino acid residues, and most preferably, about 6 to about 22 amino acids. In another embodiment, a colon and/or colon cancer related protein of the invention binding polypeptide has in the range of 15-100
10 amino acids, or 20-50 amino acids.

The selected colon cancer antigen protein of the invention binding polypeptide can be obtained by chemical synthesis or recombinant expression.

15

Targeted Delivery

In another embodiment, the invention provides a method of delivering compositions to targeted cells expressing a colon cancer antigen of the invention.

As discussed herein, polypeptides or antibodies of the invention may be associated
20 with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions. In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (including antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method
25 for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

In another embodiment, the invention provides a method for the specific destruction
30 of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention (e.g., polypeptides of the invention or antibodies of the invention) in association with toxins or cytotoxic prodrugs.

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By "toxin" is meant compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNase, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. By "cytotoxic prodrug" is meant a non-toxic compound that is converted by an enzyme, normally present in the cell, into a cytotoxic compound. Cytotoxic prodrugs that may be used according to the methods of the invention include, but are not limited to, glutamyl derivatives of benzoic acid mustard alkylating agent, phosphate derivatives of etoposide or mitomycin C, cytosine arabinoside, daunorubisin, and phenoxyacetamide derivatives of doxorubicin.

Drug Screening

Further contemplated is the use of the polypeptides of the present invention, or the polynucleotides encoding these polypeptides, to screen for molecules which modify the activities of the polypeptides of the present invention. Such a method would include contacting the polypeptide of the present invention with a selected compound(s) suspected of having antagonist or agonist activity, and assaying the activity of these polypeptides following binding.

This invention is particularly useful for screening therapeutic compounds by using the polypeptides of the present invention, or binding fragments thereof, in any of a variety of drug screening techniques. The polypeptide or fragment employed in such a test may be affixed to a solid support, expressed on a cell surface, free in solution, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide or fragment. Drugs are screened against such transformed cells in competitive binding assays. One may measure, for example, the formulation of complexes between the agent being tested and a polypeptide of the present invention.

Thus, the present invention provides methods of screening for drugs or any other agents which affect activities mediated by the polypeptides of the present invention. These methods comprise contacting such an agent with a polypeptide of the present invention or a fragment thereof and assaying for the presence of a complex between the agent and the polypeptide or a fragment thereof, by methods well known in the art. In such a competitive binding assay, the agents to screen are typically labeled. Following incubation, free agent is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of a particular agent to bind to the polypeptides of the present invention.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the polypeptides of the present invention, and is described in great detail in European Patent Application 84/03564, published on September 13, 1984, which is incorporated herein by reference herein. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with polypeptides of the present invention and washed. Bound polypeptides are then detected by methods well known in the art. Purified polypeptides are coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies may be used to capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding polypeptides of the present invention specifically compete with a test compound for binding to the polypeptides or fragments thereof. In this manner, the antibodies are used to detect the presence of any peptide which shares one or more antigenic epitopes with a polypeptide of the invention.

Antisense And Ribozyme (Antagonists)

In specific embodiments, antagonists according to the present invention are nucleic acids corresponding to the sequences contained in SEQ ID NO:X, or the complementary strand thereof, and/or to nucleotide sequences contained in the deposited clone identified in Table 1. In one embodiment, antisense sequence is generated internally, by the organism, in another embodiment, the antisense sequence is separately administered (see, for example, O'Connor, J., Neurochem. 56:560 (1991). Oligodeoxynucleotides as Antisense Inhibitors of

Gene Expression, CRC Press, Boca Raton, FL (1988). Antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are discussed for example, in Okano, J., *Neurochem.* 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance, Lee et al., *Nucleic Acids Research* 6:3073 (1979); Cooney et al., *Science* 241:456 (1988); and Dervan et al., *Science* 251:1300 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

For example, the use of c-myc and c-myb antisense RNA constructs to inhibit the growth of the non-lymphocytic leukemia cell line HL-60 and other cell lines was previously described. (Wickstrom et al. (1988); Anfossi et al. (1989)). These experiments were performed in vitro by incubating cells with the oligoribonucleotide. A similar procedure for in vivo use is described in WO 91/15580. Briefly, a pair of oligonucleotides for a given antisense RNA is produced as follows: A sequence complimentary to the first 15 bases of the open reading frame is flanked by an EcoR1 site on the 5' end and a HindIII site on the 3' end. Next, the pair of oligonucleotides is heated at 90°C for one minute and then annealed in 2X ligation buffer (20mM TRIS HCl pH 7.5, 10mM MgCl₂, 10mM dithiothreitol (DTT) and 0.2 mM ATP) and then ligated to the EcoR1/Hind III site of the retroviral vector PMV7 (WO 91/15580).

For example, the 5' coding portion of a polynucleotide that encodes the polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into receptor polypeptide.

In one embodiment, the antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the

art, used for replication and expression in vertebrate cells. Expression of the sequence encoding the polypeptide of the present invention or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, *Nature* 29:304-310 (1981), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., *Cell* 22:787-797 (1980), the herpes thymidine promoter (Wagner et al., *Proc. Natl. Acad. Sci. U.S.A.* 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster, et al., *Nature* 296:39-42 (1982)), etc.

10 The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a gene of the present invention. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the hybridizing nucleic acid, the more base mismatches with a RNA it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

20 Oligonucleotides that are complementary to the 5' end of the message, e.g., the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., 1994, *Nature* 372:333-335. Thus, oligonucleotides complementary to either the 5'- or 3'- non- translated, non-coding regions of shown in Table 1 could be used in an antisense approach to inhibit translation of endogenous mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5', 3' or coding region

of mRNA of the present invention, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

5 The polynucleotides of the invention can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors in vivo), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., 10 1989, Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556; Lemaitre et al., 1987, Proc. Natl. Acad. Sci. 84:648-652; PCT Publication No. WO88/09810, published December 15, 1988) or the blood-brain barrier (see, e.g., PCT Publication No. WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents. (See, e.g., Krol et al., 1988, BioTechniques 6:958-15 976) or intercalating agents. (See, e.g., Zon, 1988, Pharm. Res. 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

 The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil, 5-bromouracil, 20 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 25 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-30 3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide comprises at least one
5 modified phosphate backbone selected from the group including, but not limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the antisense oligonucleotide is an a-anomeric
10 oligonucleotide. An a-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual b-units, the strands run parallel to each other (Gautier et al., 1987, Nucl. Acids Res. 15:6625-6641). The oligonucleotide is a 2'-O-methylribonucleotide (Inoue et al., 1987, Nucl. Acids Res. 15:6131-6148), or a chimeric RNA-DNA analogue (Inoue et al., 1987, FEBS Lett. 215:327-330).

15 Polynucleotides of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (1988, Nucl. Acids Res. 16:3209), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer
20 supports (Sarin et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451), etc.

While antisense nucleotides complementary to the coding region sequence could be used, those complementary to the transcribed untranslated region are most preferred.

Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published October 4,
25 1990; Sarver et al, Science 247:1222-1225 (1990). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'.
30 The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, Nature 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within the nucleotide sequence of

SEQ ID NO:X. Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the mRNA; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

As in the antisense approach, the ribozymes of the invention can be composed of modified oligonucleotides (e.g. for improved stability, targeting, etc.) and should be delivered to cells which express *in vivo*. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, such as, for example, pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous messages and inhibit translation. Since ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Antagonist/agonist compounds may be employed to inhibit the cell growth and proliferation effects of the polypeptides of the present invention on neoplastic cells and tissues, i.e. stimulation of angiogenesis of tumors, and, therefore, retard or prevent abnormal cellular growth and proliferation, for example, in tumor formation or growth.

The antagonist/agonist may also be employed to prevent hyper-vascular diseases, and prevent the proliferation of epithelial lens cells after extracapsular cataract surgery. Prevention of the mitogenic activity of the polypeptides of the present invention may also be desirable in cases such as restenosis after balloon angioplasty.

The antagonist/agonist may also be employed to prevent the growth of scar tissue during wound healing.

The antagonist/agonist may also be employed to treat the diseases described herein. Thus, the invention provides a method of treating disorders or diseases, including but not limited to the disorders or diseases listed throughout this application, associated with overexpression of a polynucleotide of the present invention by administering to a patient (a) an antisense molecule directed to the polynucleotide of the present invention, and/or (b) a ribozyme directed to the polynucleotide of the present invention.

30 **Other Activities**

A polypeptide, polynucleotide, agonist, or antagonist of the present invention, as a result of the ability to stimulate vascular endothelial cell growth, may be employed in

treatment for stimulating re-vascularization of ischemic tissues due to various disease conditions such as thrombosis, arteriosclerosis, and other cardiovascular conditions. The polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed to stimulate angiogenesis and limb regeneration, as discussed above.

5 A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed for treating wounds due to injuries, burns, post-operative tissue repair, and ulcers since they are mitogenic to various cells of different origins, such as fibroblast cells and skeletal muscle cells, and therefore, facilitate the repair or replacement of damaged or diseased tissue.

10 A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed stimulate neuronal growth and to treat and prevent neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions such as Alzheimer's disease, Parkinson's disease, and AIDS-related complex. A polypeptide, polynucleotide, agonist, or antagonist of the present invention may have the ability to stimulate chondrocyte
15 growth, therefore, they may be employed to enhance bone and periodontal regeneration and aid in tissue transplants or bone grafts.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may be also be employed to prevent skin aging due to sunburn by stimulating keratinocyte growth.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may
20 also be employed for preventing hair loss, since FGF family members activate hair-forming cells and promotes melanocyte growth. Along the same lines, a polypeptide, polynucleotide, agonist, or antagonist of the present invention may be employed to stimulate growth and differentiation of hematopoietic cells and bone marrow cells when used in combination with other cytokines.

25 A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed to maintain organs before transplantation or for supporting cell culture of primary tissues. A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed for inducing tissue of mesodermal origin to differentiate in early embryos.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may
30 also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, a polypeptide, polynucleotide, agonist, or antagonist of the present invention may be used to modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may be used to change a mammal's mental state or physical state by influencing biorhythms, circadian rhythms, depression (including depressive disorders), tendency for violence, tolerance for pain, reproductive capabilities (preferably by Activin or Inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components.

The above-recited applications have uses in a wide variety of hosts. Such hosts include, but are not limited to, human, murine, rabbit, goat, guinea pig, camel, horse, mouse, rat, hamster, pig, micro-pig, chicken, goat, cow, sheep, dog, cat, non-human primate, and human. In specific embodiments, the host is a mouse, rabbit, goat, guinea pig, chicken, rat, hamster, pig, sheep, dog or cat. In preferred embodiments, the host is a mammal. In most preferred embodiments, the host is a human.

Other Preferred Embodiments

Other preferred embodiments of the claimed invention include an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 50 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5' Nucleotide of the Clone Sequence and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

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Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5' Nucleotide of the Start Codon and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 150 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

Further preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 500 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

A further preferred embodiment is a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the nucleotide sequence of SEQ ID NO:X beginning with the nucleotide at about the position of the 5' Nucleotide of the First Amino Acid and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence of SEQ ID NO:X.

Also preferred is an isolated nucleic acid molecule which hybridizes under stringent hybridization conditions to a nucleic acid molecule, wherein said nucleic acid molecule which hybridizes does not hybridize under stringent hybridization conditions to a nucleic acid molecule having a nucleotide sequence consisting of only A residues or of only T residues.

Also preferred is a composition of matter comprising a DNA molecule which comprises a human cDNA clone identified by a cDNA Clone Identifier in Table 1, which DNA molecule is contained in a cDNA library shown in Table 9 which was deposited with the American Type Culture Collection and given the ATCC Deposit Numbers shown above for said cDNA library identifier in Table 2.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in the nucleotide sequence of a human cDNA clone identified by a cDNA Clone Identifier in Table 1, which DNA molecule is contained in a cDNA library shown in Table 9 which was

deposited with the American Type Culture Collection and given the ATCC Deposit Numbers shown above for said cDNA library identifier in Table 2.

Also preferred is an isolated nucleic acid molecule, wherein said sequence of at least 50 contiguous nucleotides is included in the nucleotide sequence of the complete open reading frame sequence encoded by said human cDNA clone.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 150 contiguous nucleotides in the nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 500 contiguous nucleotides in the nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is a method for detecting in a biological sample a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Id in Table 1 which DNA molecule is contained in a cDNA library shown in Table 9 which was deposited with the American Type Culture Collection and given the ATCC Deposit Numbers shown above for said cDNA library identifier; which method comprises a step of comparing a nucleotide sequence of at least one nucleic acid molecule in said sample with a sequence selected from said group and determining whether the sequence of said nucleic acid molecule in said sample is at least 95% identical to said selected sequence.

Also preferred is the above method wherein said step of comparing sequences comprises determining the extent of nucleic acid hybridization between nucleic acid molecules in said sample and a nucleic acid molecule comprising said sequence selected from said group. Similarly, also preferred is the above method wherein said step of comparing sequences is performed by comparing the nucleotide sequence determined from a nucleic acid molecule in said sample with said sequence selected from said group. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

A further preferred embodiment is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting nucleic acid molecules in said sample, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in a cDNA library shown in Table 9 which was deposited with the American Type Culture Collection and given the ATCC Deposit Numbers shown above for said cDNA library identifier.

The method for identifying the species, tissue or cell type of a biological sample can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a protein identified in Table 1, wherein the method comprises a step of detecting in a biological sample obtained from said subject nucleic acid molecules, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in a cDNA library shown in Table 9 which was deposited with the American Type Culture Collection and given the ATCC Deposit Numbers shown above for said cDNA library identifier.

The method for diagnosing a pathological condition can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a composition of matter comprising isolated nucleic acid molecules wherein the nucleotide sequences of said nucleic acid molecules comprise a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the

group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in a cDNA library shown in Table 9 which was deposited with the American Type Culture Collection and given the ATCC Deposit
5 Numbers shown above for said cDNA library identifier. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

Also preferred is a composition of matter comprising isolated nucleic acid molecules wherein the nucleotide sequences of said nucleic acid molecules comprise a DNA microarray or "chip" of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 100, 150, 200, 250, 300,
10 500, 1000, 2000, 3000 or 4000 nucleotide sequences, wherein at least one sequence in said DNA microarray or "chip" is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and
15 contained in a cDNA library shown in Table 9 which was deposited with the American Type Culture Collection and given the ATCC Deposit Numbers shown above for said cDNA library identifier. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least
20 90% identical to a sequence of at least about 10 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1.

25 Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the complete amino acid sequence of SEQ ID NO:Y wherein Y is any
30 integer as defined in Table 1.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the complete

amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in a cDNA library shown in Table 9 which was deposited with the American Type Culture Collection and given the ATCC Deposit Numbers shown above for said cDNA library identifier.

5 Also preferred is a polypeptide wherein said sequence of contiguous amino acids is included in the amino acid sequence of a portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in a cDNA library shown in Table 9 which was deposited with the American Type Culture Collection and given the ATCC Deposit Numbers shown above for said cDNA library identifier in Table 2.

10 Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in a cDNA library shown in Table 9 which was deposited with the American Type Culture Collection and given the ATCC Deposit Numbers shown
15 above for said cDNA library identifier.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in a cDNA library shown in Table 9 which was deposited
20 with the American Type Culture Collection and given the ATCC Deposit Numbers shown above for said cDNA library identifier.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in a cDNA library shown in
25 Table 9 which was deposited with the American Type Culture Collection and given the ATCC Deposit Numbers shown above for said cDNA library identifier.

Further preferred is an isolated antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid
30 sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in a cDNA library shown in Table 9 which was

deposited with the American Type Culture Collection and given the ATCC Deposit Numbers shown above for said cDNA library identifier.

Further preferred is a method for detecting in a biological sample a polypeptide comprising an amino acid sequence which is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in a cDNA library shown in Table 9 which was deposited with the American Type Culture Collection and given the ATCC Deposit Numbers shown above for said cDNA library identifier; which method comprises a step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group and determining whether the sequence of said polypeptide molecule in said sample is at least 90% identical to said sequence of at least 10 contiguous amino acids.

Also preferred is the above method wherein said step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group comprises determining the extent of specific binding of polypeptides in said sample to an antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in a cDNA library shown in Table 9 which was deposited with the American Type Culture Collection and given the ATCC Deposit Numbers shown above for said cDNA library identifier.

Also preferred is the above method wherein said step of comparing sequences is performed by comparing the amino acid sequence determined from a polypeptide molecule in said sample with said sequence selected from said group.

Also preferred is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting polypeptide molecules in said sample, if any, comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an

amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in a cDNA library shown in Table 9 which was deposited with the American Type Culture Collection and given the
5 ATCC Deposit Numbers shown above for said cDNA library identifier.

Also preferred is the above method for identifying the species, tissue or cell type of a biological sample, which method comprises a step of detecting polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10
10 contiguous amino acids in a sequence selected from the above group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a protein identified in Table 1, which method comprises a step of detecting in a biological sample obtained from said subject polypeptide molecules comprising an amino acid sequence in a panel of at least
15 two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in a cDNA
20 library shown in Table 9 which was deposited with the American Type Culture Collection and given the ATCC Deposit Numbers shown above for said cDNA library identifier.

In any of these methods, the step of detecting said polypeptide molecules includes using an antibody.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence
25 which is at least 95% identical to a nucleotide sequence encoding a polypeptide wherein said polypeptide comprises an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a
30 cDNA Clone Identifier in Table 1 and contained in a cDNA library shown in Table 9 which was deposited with the American Type Culture Collection and given the ATCC Deposit Numbers shown above for said cDNA library identifier.

Also preferred is an isolated nucleic acid molecule, wherein said nucleotide sequence encoding a polypeptide has been optimized for expression of said polypeptide in a prokaryotic host.

Also preferred is an isolated nucleic acid molecule, wherein said polypeptide
5 comprises an amino acid sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in a cDNA library shown in Table 9 which was deposited with the American Type Culture Collection and given the ATCC Deposit Numbers
10 shown above for said cDNA library identifier.

Further preferred is a method of making a recombinant vector comprising inserting any of the above isolated nucleic acid molecule into a vector. Also preferred is the recombinant vector produced by this method. Also preferred is a method of making a recombinant host cell comprising introducing the vector into a host cell, as well as the
15 recombinant host cell produced by this method.

Also preferred is a method of making an isolated polypeptide comprising culturing this recombinant host cell under conditions such that said polypeptide is expressed and recovering said polypeptide. Also preferred is this method of making an isolated polypeptide, wherein said recombinant host cell is a eukaryotic cell and said polypeptide is a
20 human protein comprising an amino acid sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y beginning with the residue at the position of the First Amino Acid of the Protein of SEQ ID NO:Y wherein Y is an integer set forth in Table 1 and said position of the First Amino Acid of the Protein of SEQ ID NO:Y is defined in Table 1; and an amino acid sequence of a protein encoded by a human cDNA clone identified by a
25 cDNA Clone Identifier in Table 1 and contained in a cDNA library shown in Table 9 which was deposited with the American Type Culture Collection and given the ATCC Deposit Numbers shown above for said cDNA library identifier.

Also preferred is a method of treatment of an individual in need of an increased level of a protein activity, which method comprises administering to such an individual a
30 Therapeutic comprising an amount of an isolated polypeptide, polynucleotide, immunogenic fragment or analogue thereof, binding agent, antibody, or antigen binding fragment of the claimed invention effective to increase the level of said protein activity in said individual.

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Also preferred is a method of treatment of an individual in need of a decreased level of a protein activity, which method comprises administering to such an individual a Therapeutic comprising an amount of an isolated polypeptide, polynucleotide, immunogenic fragment or analogue thereof, binding agent, antibody, or antigen binding
5 fragment of the claimed invention effective to decrease the level of said protein activity in said individual.

Also preferred is a method of treatment of an individual in need of a specific delivery of toxic compositions to diseased cells (e.g., including, but not limited to, colon or colon cancer cells or tissues), which method comprises administering to such an individual a
10 Therapeutic comprising an amount of an isolated polypeptide of the invention, including, but not limited to a binding agent, or antibody of the claimed invention that are associated with toxin or cytotoxic prodrugs.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not
15 intended as limiting.

Examples

20

Example 1: Isolation of a Selected cDNA Clone From the Deposited Sample

Each cDNA clone in a cited ATCC deposit is contained in a plasmid vector. Table 9 identifies the vectors used to construct the cDNA library from which each clone was isolated.

Table 9.

LIBRARIES DEPOSITED	VECTOR	ATCC DEPOSIT NO.
HASA	Uni-ZAP XR	LP03
HFCA HFCD HFCE HFCF	Uni-ZAP XR	LP13
HFKF	Uni-ZAP XR	LP13
HE8A HE8B HE8C HE8D HE8E HE8F HE8N HE8O HE8P HE8Q HE8T HE8U	Uni-ZAP XR	LP03
HGBA HGBG HGBH	Uni-ZAP XR	LP13
HGBB	Uni-ZAP XR	LP03
HHFA	pBluescript	NA
HLHA HLHB HLHC HLHD HLHE HLHG	Uni-ZAP XR	LP03
HOOA	pBluescript	NA
HPLB	Uni-ZAP XR	NA
HPMD HPME HPMF	Uni-ZAP XR	LP03
HPRA	Uni-ZAP XR	LP13
HSIA HSIC HSID HSIE	Uni-ZAP XR	LP03
HTEA HTEB HTEC HTED HTEE HTEF HTEG HTEH HTEJ HTEK	Uni-ZAP XR	LP13
HTPA HTPC	Uni-ZAP XR	LP03
HTTB HTTC HTTD HTTE HTTF	Uni-ZAP XR	LP13
HAPA HAPC	Uni-ZAP XR	LP03
HETA HETB HETC HETD HETG HETH HETI HETJ	Uni-ZAP XR	LP03
HHFB HHFC HHFG HHFH HHFI	Uni-ZAP XR	LP13
HHPE HHPG	Uni-ZAP XR	LP03
HCE1 HCE2 HCE3 HCE4 HCEC HCED HCEE HCEF HCEI HCEM HCEN HCEO HCEP	Uni-ZAP XR	LP03
HUVC HUVD	Uni-ZAP XR	LP13
HUKB HUKF	Lamda ZAP II	LP13
HTHC HTHD	Uni-ZAP XR	LP13
HSTA	Uni-ZAP XR	LP13
HTAE	Uni-ZAP XR	LP13
HLEA	Uni-ZAP XR	PA005 Phage
HFEA HFEB	Uni-ZAP XR	LP13
HJPA HJPC	Uni-ZAP XR	LP13
HCNA	Lambda ZAP II	LP01
HTSG	pBS	LP05
HLTA HLTB HLTC HLTD HLTE	Uni-ZAP XR	LP03
HAHS	pBluescript	LP13
HALS	Uni-ZAP XR	LP13
HE6B HE6F HE6G	Uni-ZAP XR	LP04
HF6S	pBluescript	LP13
HPMS	pBluescript	LP03
HTYS	pBluescript	NA
HRDB HRDD HRDE HRDF	Uni-ZAP XR	LP03
HCAB	Uni-ZAP XR	LP13
HL3A	Uni-ZAP XR	PA005 Phage
HRGD	Uni-ZAP XR	LP13
HSSE HSSG HSSI	Uni-ZAP XR	LP04
HSUA HSUB	Uni-ZAP XR	LP03
HT3A	Uni-ZAP XR	NA

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LIBRARIES DEPOSITED	VECTOR	ATCC DEPOSIT NO.
HT4C	Uni-ZAP XR	LP03
HE9F HE9H HE9M HE9N HE9O HE9P HE9Q HE9R HE9S HE9T	Uni-ZAP XR	LP13
HEPA HEPB	Uni-ZAP XR	LP04
HSFA	Uni-ZAP XR	LP13
HATA HATB HATC HATE	Uni-ZAP XR	LP13
HT3B	Uni-ZAP XR	PA005 Phage
HSNA	Uni-ZAP XR	LP04
HPFC	Uni-ZAP XR	LP04
HE2A HE2D HE2E HE2H HE2I HE2O	Uni-ZAP XR	LP13
HE2B HE2C HE2F HE2P	Uni-ZAP XR	LP13
HCBB	Uni-ZAP XR	NA
HFGA	Uni-ZAP XR	LP03
HNEA HNED	Uni-ZAP XR	LP13
HBGB	Uni-ZAP XR	LP03
HKCA	Uni-ZAP XR	PA005 Phage
HKLA	Lambda ZAP II	PA005 Phage
HBNA	Uni-ZAP XR	LP03
HCET	pBluescript	PA005 Phage
HKCS HKCU	pBluescript	LP03
HKCT	pBluescript	PA005 Phage
HLIS	pBluescript	LP13
HLHS HLHT	pBluescript	LP13
HPRT	pBluescript	PA005 Phage
HPTT	Uni-ZAP XR	LP13
HRGS	pBluescript	LP03
HSUS	pBluescript	LP13
HT2S	Uni-ZAP XR	NA
HCNS	pBluescript	PA005 Phage
HCNU	pBluescript	PA005 Phage
HKLR	pBluescript	PA005 Phage
HKLS	pBluescript	PA005 Phage
HKTA	Uni-ZAP XR	PA005 Phage
HHFU	pBluescript	NA
HE8S	Uni-ZAP XR	LP03
HCDC HCDE	Uni-ZAP XR	LP03
HOAA	Uni-ZAP XR	LP13
HTLA HTLD HTLE	Uni-ZAP XR	LP03
HLMD	Uni-ZAP XR	PA005 Phage
HLMI HLMM	Lambda Zap II	LP01

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LIBRARIES DEPOSITED	VECTOR	ATCC DEPOSIT NO.
H6EA H6EB	Uni-ZAP XR	LP03
HCEV HCEY	Uni-ZAP XR	LP03
HCQA HCQB	Lambda Zap II	LP01
HTOA HTOD HTOH HTOJ	Uni-ZAP XR	LP13
HTXC HTXF	Uni-ZAP XR	LP03
HMEC HMEE HMEG HMEI HMEK	Lambda Zap II	LP01
HMEB	Lambda Zap II	LP13
HNFE HNFF HNFG HNFH	Uni-ZAP XR	LP03
HKEA	ZAP express	PA005 Phage
HMGB	Uni-ZAP XR	LP13
HMHB	Uni-ZAP XR	PA005 Phage
HAUA HAUB	Uni-ZAP XR	LP13
HAQB	Uni-ZAP XR	LP13
HCWH	ZAP express	LP02
HCUC	ZAP express	LP02
HSVB HSVC	Uni-ZAP XR	LP03
HPXA	pBluescript	NA
HBJE HBJF HBJJ HBJM	Uni-ZAP XR	LP13
HCRB	Uni-ZAP XR	LP03
HODA HODB HODC HODD	Uni-ZAP XR	LP13
HDSA	Uni-ZAP XR	LP03
HLQA HLQB	Lambda Zap II	LP01
HHGC HHGD	Lambda Zap II	LP01
HCPA	Uni-ZAP XR	LP13
HMWA HMWB HMWD HMWF HMWH HMWI	Uni-ZAP XR	LP03
HERA	Uni-ZAP XR	LP13
HGLA	Uni-ZAP XR	LP13
HWTB HWTC	Uni-ZAP XR	LP13
HLLC	pCMVSPORT1	PA005 DNA
HLIB HLIC	pCMVSPORT1	LP12
HKDB	pCMVSPORT1	NA
HRKA	pBluescript	PA005 Phage
HOSX	pBluescript	PA005 Phage
HEAA	Uni-ZAP XR	LP13
HBCB HBCC	Uni-ZAP XR	LP21
HHBE HHBF HBBH	pCMVSPORT1	LP12
HBBB	pCMVSPORT1	LP12
HLJB HLJD HLJE	pCMVSPORT1	LP12
HSEB	pCMVSPORT1	NA
HNAA	pSPORT1	NA
HBSA	Uni-ZAP XR	LP04
HBBM	pCMVSPORT1	NA
HADM	pBluescript	NA
HMKA HMKC	pSPORT1	LP12
HFVH HFVI HFVJ HFVK	pBluescript	LP03
HKIM	Lambda Zap II	PA005 Phage

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LIBRARIES DEPOSITED	VECTOR	ATCC DEPOSIT NO.
HCUD HCUE HCUG	ZAP express	LP02
HKIS	pBluescript	NA
HSDS	pBluescript	LP13
HBAG HBAH	pSport1	NA
HUSG HUSI HUSJ	pSport1	LP10
HUSX HUSY HUSZ	pSport1	LP10
HOFM	pCMVSPORT 2.0	LP07
HNFI	pBluescript	LP03
HBMC HBMD	pBluescript	LP03
HCFB HCFC HCFD	pSport1	LP12
HCFL HCFM HCFN HCFO	pSport1	LP12
HPTW	pBluescript	PA005 Phage
HADC HADF	pSport1	LP10
HOVA HOVC HOVD HOVE	pSport1	LP10
HKML HKMM	pBluescript	LP03
HUSF	pBluescript	NA
HOGA HOGB HOGC HOGD HOGE	pCMVSPORT 2.0	LP12
HTWB HTWC HTWD HTWE HTWF	pSport1	LP10
HBXF	ZAP express	LP02
HEOA	pBluescript	PA005 DNA
HSDX	pBluescript	LP13
HMMA	pSport1	LP12
HLA HLYB HLYC HLYD HLYE HLYG	pSport1	LP10
HCGL	pCMVSPORT 2.0	LP07
HSDZ	pBluescript	LP13
HEON HEOQ HEOS	pSport1	LP10
HCEB	pSport1	LP10
HADT	pBluescript	NA
HTDA	pSport1	LP12
HSPA HSPB	pSport1	LP10
HSPM	pSport1	LP10
HCHA HCHB HCHC	pSport1	LP10
HCHM HCHO	pSport1	LP10
HDLA	pCMVSPORT 2.0	LP07
HDTA HDTB HDTD HDTE HDTG HDTH HDTI HDTJ HDTK HDTL HDTM	pCMVSPORT 2.0	LP07
HTJM HTJN	pCMVSPORT 2.0	LP12
HCIA	pSport1	LP10
H6BS	Uni-ZAP XR	LP03
HKAA HKAB HKAC HKAD HKAE HKAF HKAH KKAJ HKAK HKAQ	pCMVSPORT 2.0	LP07
HDAA HDAB HDAC	pSport1	LP10
HUFA HUFB HUFC HUFD HUFF	pSport1	LP10
HLDB HLDC HLDD	pCMVSPORT 3.0	LP08

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LIBRARIES DEPOSITED	VECTOR	ATCC DEPOSIT NO.
HLDN HLDO	pCMVSPORT 3.0	LP08
HNDA	pCMVSPORT 2.0	LP07
HMTA HMTB	pCMVSPORT 3.0	LP08
HNTA HNTB HNTC HNTD HNTE	pCMVSPORT 3.0	LP08
HNTM	pSPORT1	LP10
HDPA HDPB HDPC HDPF HDPG HDPH HDPI HDPJ HDPK HDPL HDPR HDPS HDPT HDPV HDPW HDPX HDQD HDQE HDQF HDQG HDQH	pCMVSPORT 3.0	LP08
HDPM HDPO HDPP HDPQ HDQP	pCMVSPORT 3.0	LP08
HMTM	PCR11	LP09
HLDX	pSPORT1	LP10
HMUB	pCMVSPORT 3.0	LP08
HULA HULC	pSPORT1	LP10
HFNA	pSPORT1	LP10
HKGA HKGB HKGC HKGD	pSPORT1	LP10
HISA HISB HISC HISD HISE	pSPORT1	LP10
HLSA	pSPORT1	LP10
HHEA HHEB HHEC HHED HHEE HHEF HHEG HHEH HHEI HHEJ	pCMVSPORT 3.0	LP08
HHEM HHEN HHEP HHEQ HHER HHET HHEU HHEV HHEW HHEX HHEY HHEZ	pCMVSPORT 3.0	LP08
HEQA	pCMVSPORT 3.0	LP08
HJMA HJMB	pCMVSPORT 3.0	LP08
HSWB	pCMVSPORT 3.0	LP08
HNTR HNTS HNTT	pSPORT1	NA
HEEA	Uni-ZAP XR	NA
HEGA	Uni-ZAP XR	NA
HSYA HSYB HSYD HSYE	pCMVSPORT 3.0	LP08
HLWA HLWB HLWC	pCMVSPORT 3.0	LP08
HRAA HRAB HRAC HRAE	pCMVSPORT 3.0	LP08
HTXJ HTXK HTXL HTXM HTXO HTXP HTXQ HTXR HTXS	Uni-ZAP XR	LP03
H6ED	Uni-ZAP XR	LP03
HAMF HAMG	pCMVSPORT 3.0	LP12
HAJA HAJB	pCMVSPORT 3.0	LP12
HDFU	pCMVSPORT 2.0	NA
HDHE	pCMVSPORT 2.0	NA
HLQD HLQE HLQF	Lamda ZAP II	LP13

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LIBRARIES DEPOSITED	VECTOR	ATCC DEPOSIT NO.
HAPN HAPO HAPQ HAPR	Uni-ZAP XR	LP13
HWBA HWBB HWBC HWBD HWBE HWBF	pCMVSPORT 3.0	LP12
HWAA HWAB HWAC HWAD HWAG HWAI	pCMVSPORT 3.0	LP12
HYAA HYAB HYAC	pCMVSPORT 3.0	LP12
HWHG HWHH	pCMVSPORT 3.0	LP12
HWHP HWHQ	pCMVSPORT 3.0	LP12
HCWU	ZAP Exress	LP13
HSIF HSIG	Uni-ZAP XR	PA005 Phage
HLTG HLTH HLTJ	Uni-ZAP XR	LP13
HARM HARN	pCMVSPORT 3.0	LP12
HBIM HBIN HBIO HBIP	pCMVSPORT 3.0	LP12
HSOB HSOD	Uni-ZAP XR	LP03
HCQC HCQD	Lambda ZAP II	LP01
HCNC HCND	Lambda ZAP II	LP01
HROB HROD	Uni-ZAP XR	LP03
HAHC	Uni-ZAP XR	LP13
HWDA	pCMVSPORT 3.0	LP12
HODE HODF HODG	Uni-ZAP XR	LP03
HTEL HTEP	Uni-ZAP XR	LP03
HGBM HGBN	Uni-ZAP XR	LP03
HTLG HTLH	Uni-ZAP XR	LP03
HHFJ HHFL HHFM	Uni-ZAP XR	LP03
HFKH HFKI HFKM	Uni-ZAP XR	LP03
HTPF HTPG HTPH HTPJ	Uni-ZAP XR	LP03
HUVF HUVG HUVH	Uni-ZAP XR	LP03
HE2J HE2L HE2R HE2T	Uni-ZAP XR	LP04
HS2A	pSport1	LP16
HS2S	pSport1	LP16
HLQG	Lambda Zap II	LP01
HA5A HA5B	pSport1	LP16
HTTI HTTK	Uni-ZAP XR	LP03
HTAH	Uni-ZAP XR	LP03
HDDN	pSport1	LP22
HPCI	Lambda Zap- CMV XR	LP21
HPCR	Lambda Zap- CMV XR	LP22
HPMK HPML	Uni-ZAP XR	LP03
HHFO	Uni-ZAP XR	LP03
HAAA	pSport1	LP22
HOOH	pSport1	LP22
HIDA	pSport1	LP22
HNOA	pSport1	LP22

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LIBRARIES DEPOSITED	VECTOR	ATCC DEPOSIT NO.
HUUA	pTrip1 Ex2	LP22
HPDO	pSport1	PA005 DNA
HPCO	pSport1	PA005 DNA
HOCM	pSport1	PA005 DNA
HNBT	pSport1	PA005 DNA
HBCJ	pSport1	PA005 DNA
HSAM	pSport1	PA005 DNA
HFXA HFXH	Lambda ZAP II	LP01
HMSA HMSC HMSD HMSF HMSG HMSH HMSI HMSJ	Uni-ZAP XR	LP03
HOSA HOSB HOSD HOSM HOSN HOSO HOSP	Uni ZAP XR	LP04
HEBA HEBB HEBF HEBG	Uni ZAP XR	NA
HAGB HAGD HAGE HAGF	Uni-ZAP XR	LP13
HSRA HSRB	Uni-ZAP XR	LP03
HPVA	Uni ZAP XR	PA005 Phage
HKIA	Uni ZAP XR	PA005 Phage
HKMA	Uni ZAP XR	NA
HSRF	Uni-ZAP XR	LP03
HSQD HSQF	Uni-ZAP XR	LP03
HSKE HSKZ	Uni-ZAP XR	LP03
HSLE HSLF HSLG HSLH	Uni-ZAP XR	LP03
HSDE HSDH	Uni-ZAP XR	LP03
HSXA HSXB HSXD	Uni-ZAP XR	LP04
HSHA HSHB	Uni-ZAP XR	LP13
HBXA HBXB HBXC	ZAP Express	LP13
HOUA HOUD	Uni-ZAP XR	LP04
HPWA HPWB HPWC	Uni-ZAP XR	LP13
HELB HELG HELH	Uni-ZAP XR	LP04
HEMF HEMG	Uni-ZAP XR	LP04
HBIB	Uni-ZAP XR	LP04
HFRA HFRB	Uni ZAP XR	PA005 Phage
HHSB HHSD	Uni-ZAP XR	LP04
HNGB HNGE HNGG HNGI	Uni-ZAP XR	LP04
HNHD HNHE HNHH	Uni-ZAP XR	LP04
HADB	Uni ZAP XR	NA
HSAX HSAW HSAX HSAZ	Uni-ZAP XR	LP04
HBMS HBMT HBMV HBMX	Uni-ZAP XR	LP04
HOBA	pBluescript	PA005 Phage
HOEE HOEF HOEK HOEL HOEM HOEN HOEO	Uni ZAP XR	PA005 Phage
HAIB HAIC HAID	Uni-ZAP XR	LP04
HTGA HTGB	Uni-ZAP XR	LP04
HEIB HEIC	Uni ZAP XR	NA

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LIBRARIES DEPOSITED	VECTOR	ATCC DEPOSIT NO.
HMCD	Uni-ZAP XR	LP04
HPCA	Uni ZAP XR	NA
HPHA	Uni-ZAP XR	LP04
HP1A HP1C	Uni-ZAP XR	LP13
HP1A HP1B HP1C HP1E	Uni-ZAP XR	LP13
HF1A HF1B HF1C	pSport1	LP10
HF1H HF1I HF1J	pSport1	LP10
HF1U	pSport1	LP10
HSKX	pBluescript	LP03
HGCO	pSport1	NA
HMVA HMVB HMVC HMVD	pSport1	LP10
HOSE HOSF	Uni-ZAP XR	LP04
HNHN HNHO	Uni ZAP XR	LP04
HTGE HTGF	Uni-ZAP XR	LP04
HFPB HFPC HFPE HFPP HFPH HFPI HFPJ HFPK	Uni-ZAP XR	LP03
HFIX HFIY HFIZ	pSport1	LP10
HOHA HOHB HOHC HOHE	pCMVSPORT 2.0	LP07
HSDJ HSDK	Uni-ZAP XR	LP03
HFOX HFOY	pSport1	LP10
HMAH HMAJ HMAK HMAM	Uni-ZAP XR	LP04
HACB HACC	Uni-ZAP XR	LP04
HFXK	Lambda ZAP II	PA005 Phage
HFAT	Uni ZAP XR	PA005 Phage
HANG	pSport1	NA
HOUH	Uni ZAP XR	NA
HMCF HMCB HMCH HMCJ	Uni-ZAP XR	LP13
HWLE HWLF HWLG HWLH HWMA	pSport1	LP14
HCRM HCRN HCRP HCRQ	pSport1	LP14
HWLI HWLJ HWLK HWLL HWMF	pSport1	LP14
HWLQ HWLR HWLU HWLV HWLW HWLX	pSport1	LP14
HBOD HBOE	pSport1	LP14
HBKD	pSport1	LP14
HWLA HWLC HWLD HWLP	pSport1	LP14
HWLM HWLN HWLO HWMB HWMC	pSport1	LP14
HVAA	pSport1	LP12
HBWC	ZAP express	LP13
HHSF HHSG	Uni ZAP XR	LP04
HSLJ	Uni ZAP XR	NA
HAQN	pSport1	LP14
HASM	pSport1	LP14
HCDM	pSport1	LP14
HFDM	pSport1	LP14
HGAM	pSport1	LP14
HHMM	pSport1	LP14
HAVM	pT-Adv	LP14
HAUT	pT-Adv	LP14
HHAT HHAU	pT-Adv	LP14
HUCN HUCO HUCP HUCQ	pSport1	LP20
HHAO	pCMVSPORT	LP15

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LIBRARIES DEPOSITED	VECTOR	ATCC DEPOSIT NO.
	3.0	
HTFN	pSport1	LP16
HMSM HMSO HMSP	Uni ZAP XR	PA005 Phage
HEPN	pSport1	LP20
HPSN	pSport1	LP20
HNSA	pSport1	LP20
HNSM	pSport1	LP20
HOCN	pSport1	LP20
HOCT	pSport1	LP20
HLXN	pSport1	LP20
HTYN	pSport1	LP20
HZAA	pSport1	LP20
HINA	pSport1	LP16
HRMA	pSport1	LP16
HSKI HSKJ HSKK	pBluescript	LP03
HACA	Uni-ZAP XR	LP13
HFAA HFAC HFAD	Uni-ZAP XR	LP04
HFAM	Uni-ZAP XR	LP04
HMIA HMIB	Uni-ZAP XR	LP04
HILB HILC	pBluescript SK-	PA005 Phage
HPBE	pBluescript SK-	LP13
HIBC HIBE	Other	NA
HPDD	pBluescript SK-	NA
HSAA HSAB HSAC	pBluescript	LP05
HSBA	pBluescript SK-	LP13
HJAA HJAC	pBluescript SK-	LP13
HJBA HJBC	pBluescript SK-	LP13
HAFB	pBS	LP05
HTNA HTNB	pBluescript SK-	LP13
HONA	pBluescript	LP05
HBMA	pBluescript SK-	NA
HARA	pBluescript	LP05
H2CA	pBluescript SK-	NA
H2MA	pBluescript SK-	NA
H2MB H2MC	pBluescript SK-	PA005 Phage
H2CB	pBluescript SK-	PA005 Phage
HCYA	pBluescript SK-	NA
HCYB	pBluescript	PA005

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LIBRARIES DEPOSITED	VECTOR	ATCC DEPOSIT NO.
	SK-	Phage
H2LA H2LB	pBluescript SK-	PA005 Phage

In many cases, the vector used to construct the library is a phage vector from which a plasmid has been excised. The table immediately below correlates the related plasmid for each phage vector used in constructing the cDNA library. For example, where a particular clone is identified in Table 1 as being isolated in the vector "Lambda Zap," the corresponding deposited clone is in "pBluescript."

	<u>Vector Used to Construct Library</u>	<u>Corresponding Deposited Plasmid</u>
	Lambda Zap	pBluescript (pBS)
	Uni-Zap XR	pBluescript (pBS)
10	Zap Express	pBK
	lafmid BA	plafmid BA
	pSport1	pSport1
	pCMVSPORT 2.0	pCMVSPORT 2.0
	pCMVSPORT 3.0	pCMVSPORT 3.0
15	pCR [®] 2.1	pCR [®] 2.1

Vectors Lambda Zap (U.S. Patent Nos. 5,128,256 and 5,286,636), Uni-Zap XR (U.S. Patent Nos. 5,128, 256 and 5,286,636), Zap Express (U.S. Patent Nos. 5,128,256 and 5,286,636), pBluescript (pBS) (Short, J. M. et al., Nucleic Acids Res. 16:7583-7600 (1988); Altling-Mees, M. A. and Short, J. M., Nucleic Acids Res. 17:9494 (1989)) and pBK (Altling-Mees, M. A. et al., Strategies 5:58-61 (1992)) are commercially available from Stratagene Cloning Systems, Inc., 11011 N. Torrey Pines Road, La Jolla, CA, 92037. pBS contains an ampicillin resistance gene and pBK contains a neomycin resistance gene. Both can be transformed into E. coli strain XL-1 Blue, also available from Stratagene. pBS comes in 4 forms SK+, SK-, KS+ and KS. The S and K refers to the orientation of the polylinker to the T7 and T3 primer sequences which flank the polylinker region ("S" is for SacI and "K" is for KpnI which are the first sites on each respective end of the linker). "+" or "-" refer to the orientation of the fl origin of replication ("ori"), such that in one orientation, single stranded rescue initiated from the fl ori generates sense strand DNA and in the other, antisense.

Vectors pSport1, pCMVSPORT 2.0 and pCMVSPORT 3.0, were obtained from Life Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors contain an ampicillin resistance gene and may be transformed into E. coli strain DH10B, also available from Life Technologies. (See, for instance, Gruber, C. E., et al., Focus 15:59 (1993).)

Vector lafmid·BA (Bento Soares, Columbia University, NY) contains an ampicillin resistance gene and can be transformed into E. coli strain XL-1 Blue. Vector pCR[®]2.1, which is available from Invitrogen, 1600 Faraday Avenue, Carlsbad, CA 92008, contains an ampicillin resistance gene and may be transformed into E. coli strain DH10B, available from
5 Life Technologies. (See, for instance, Clark, J. M., Nuc. Acids Res. 16:9677-9686 (1988) and Mead, D. et al., Bio/Technology 9: (1991).) Preferably, a polynucleotide of the present invention does not comprise the phage vector sequences identified for the particular clone in Table 1, as well as the corresponding plasmid vector sequences designated above.

The deposited material in the sample assigned the ATCC Deposit Number cited in
10 Table 2 for any given cDNA clone also may contain one or more additional plasmids, each comprising a cDNA clone different from that given clone. Thus, deposits sharing the same ATCC Deposit Number contain at least a plasmid for each cDNA clone identified in Table 1. Typically, each ATCC deposit sample cited in Table 2 comprises a mixture of approximately equal amounts (by weight) of about 50 plasmid DNAs, each containing a different cDNA
15 clone; but such a deposit sample may include plasmids for more or less than 50 cDNA clones, up to about 500 cDNA clones.

Two approaches can be used to isolate a particular clone from the deposited sample of plasmid DNAs cited for that library in Table 2 and 9. First, a plasmid is directly isolated by screening the libraries using a polynucleotide probe corresponding to SEQ ID NO:X.

20 Particularly, a specific polynucleotide with 30-40 nucleotides is synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported. The oligonucleotide is labeled, for instance, with ³²P-γ-ATP using T4 polynucleotide kinase and purified according to routine methods. (E.g., Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring, NY (1982).) The plasmid
25 mixture is transformed into a suitable host, as indicated above (such as XL-1 Blue (Stratagene)) using techniques known to those of skill in the art, such as those provided by the vector supplier or in related publications or patents cited above. The transformants are plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate. These plates are screened using
30 Nylon membranes according to routine methods for bacterial colony screening (e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Edit., (1989), Cold Spring

Harbor Laboratory Press, pages 1.93 to 1.104), or other techniques known to those of skill in the art.

Alternatively, two primers of 17-20 nucleotides derived from both ends of the SEQ ID NO:X (i.e., within the region of SEQ ID NO:X bounded by the 5' NT and the 3' NT of the clone defined in Table 1) are synthesized and used to amplify the desired cDNA using the deposited cDNA plasmid as a template. The polymerase chain reaction is carried out under routine conditions, for instance, in 25 μ l of reaction mixture with 0.5 μ g of the above cDNA template. A convenient reaction mixture is 1.5-5 mM $MgCl_2$, 0.01% (w/v) gelatin, 20 μ M each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of PCR (denaturation at 94°C for 1 min; annealing at 55°C for 1 min; elongation at 72°C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the selected sequence by subcloning and sequencing the DNA product.

Several methods are available for the identification of the 5' or 3' non-coding portions of a gene which may not be present in the deposited clone. These methods include but are not limited to, filter probing, clone enrichment using specific probes, and protocols similar or identical to 5' and 3' "RACE" protocols which are well known in the art. For instance, a method similar to 5' RACE is available for generating the missing 5' end of a desired full-length transcript. (Fromont-Racine et al., *Nucleic Acids Res.* 21(7):1683-1684 (1993).)

Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcripts. A primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest is used to PCR amplify the 5' portion of the desired full-length gene. This amplified product may then be sequenced and used to generate the full length gene.

This above method starts with total RNA isolated from the desired source, although poly-A⁺ RNA can be used. The RNA preparation can then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase should then be inactivated and the RNA treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of

the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

This modified RNA preparation is used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction is used as a template
5 for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the desired gene.

10 ***Example 2: Isolation of Genomic Clones Corresponding to a Polynucleotide***

A human genomic P1 library (Genomic Systems, Inc.) is screened by PCR using primers selected for the cDNA sequence corresponding to SEQ ID NO:X., according to the method described in Example 1. (See also, Sambrook.)

15

Example 3: Tissue specific expression analysis

The Human Genome Sciences, Inc. (HGS) database is derived from sequencing tissue specific cDNA libraries. Libraries generated from a particular tissue (e.g., those shown in
20 Table 3 and 5) are selected and the specific tissue expression pattern of EST groups or assembled contigs within these libraries is determined by comparison of the expression patterns of those groups or contigs within the entire database. ESTs which are predicted to have significantly enhances expression in colon or colon cancer tissues were selected.

The original clone from which the specific EST sequence was generated, is obtained
25 from the catalogued library of clones and the insert amplified by PCR using methods known in the art. The PCR product is denatured then transferred in 96 well format to a nylon membrane (Schleicher and Scheull) generating an array filter of colon and/or colon cancer related clones. Housekeeping genes, maize genes, known tissue specific genes and known membrane localized class I genes are included on the filters as controls. These targets can be
30 used in signal normalization and to validate assay sensitivity. Additional targets are included to monitor probe length and specificity of hybridization.

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Radioactively labeled hybridization probes are generated by first strand cDNA synthesis per the manufacturer's instructions (Life Technologies) from mRNA/RNA samples prepared from the specific tissue being analyzed. The hybridization probes are purified by gel exclusion chromatography, quantitated, and hybridized with the array filters in hybridization bottles at 65°C overnight. The filters are washed under stringent conditions and signals are captured using a Fuji phosphorimager.

Data is extracted using AIS software and following background subtraction, signal normalization is performed. This includes a normalization of filter-wide expression levels between different experimental runs. Genes that are differentially expressed in the tissue of interest are identified and the full length sequence of these clones is generated.

Example 4: Chromosomal Mapping of the Polynucleotides

An oligonucleotide primer set is designed according to the sequence at the 5' end of SEQ ID NO:X. This primer preferably spans about 100 nucleotides. This primer set is then used in a polymerase chain reaction under the following set of conditions : 30 seconds, 95°C; 1 minute, 56°C; 1 minute, 70°C. This cycle is repeated 32 times followed by one 5 minute cycle at 70°C. Human, mouse, and hamster DNA is used as template in addition to a somatic cell hybrid panel containing individual chromosomes or chromosome fragments (Bios, Inc). The reactions is analyzed on either 8% polyacrylamide gels or 3.5 % agarose gels. Chromosome mapping is determined by the presence of an approximately 100 bp PCR fragment in the particular somatic cell hybrid.

Example 5: Bacterial Expression of a Polypeptide

A polynucleotide encoding a polypeptide of the present invention is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, as outlined in Example 1, to synthesize insertion fragments. The primers used to amplify the cDNA insert should preferably contain restriction sites, such as BamHI and XbaI, at the 5' end of the primers in order to clone the amplified product into the expression vector. For example, BamHI and XbaI correspond to the restriction enzyme sites on the bacterial

expression vector pQE-9. (Qiagen, Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning sites.

5 The pQE-9 vector is digested with BamHI and XbaI and the amplified fragment is ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the E. coli strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to
10 grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical
15 density 600 (O.D.⁶⁰⁰) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression.

Cells are grown for an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic agent 6 Molar
20 Guanidine HCl by stirring for 3-4 hours at 4°C. The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist (1995) QIAGEN, Inc., *supra*).

25 Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is eluted with 6 M guanidine-HCl, pH 5.

The purified protein is then renatured by dialyzing it against phosphate-buffered
30 saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500

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mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins are eluted by the addition of 250 mM imidazole. Imidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl.

5 The purified protein is stored at 4° C or frozen at -80° C.

In addition to the above expression vector, the present invention further includes an expression vector comprising phage operator and promoter elements operatively linked to a polynucleotide of the present invention, called pHE4a. (ATCC Accession Number 209645, deposited on February 25, 1998.) This vector contains: 1) a neomycinphosphotransferase
10 gene as a selection marker, 2) an *E. coli* origin of replication, 3) a T5 phage promoter sequence, 4) two lac operator sequences, 5) a Shine-Delgarno sequence, and 6) the lactose operon repressor gene (*lacIq*). The origin of replication (*oriC*) is derived from pUC19 (LTI, Gaithersburg, MD). The promoter sequence and operator sequences are made synthetically.

DNA can be inserted into the pHEa by restricting the vector with NdeI and XbaI,
15 BamHI, XhoI, or Asp718, running the restricted product on a gel, and isolating the larger fragment (the stuffer fragment should be about 310 base pairs). The DNA insert is generated according to the PCR protocol described in Example 1, using PCR primers having restriction sites for NdeI (5' primer) and XbaI, BamHI, XhoI, or Asp718 (3' primer). The PCR insert is gel purified and restricted with compatible enzymes. The insert and vector are ligated
20 according to standard protocols.

The engineered vector could easily be substituted in the above protocol to express protein in a bacterial system.

Example 6: Purification of a Polypeptide from an Inclusion Body

25

The following alternative method can be used to purify a polypeptide expressed in *E. coli* when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10°C.

Upon completion of the production phase of the *E. coli* fermentation, the cell culture
30 is cooled to 4-10°C and the cells harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by

weight, is suspended in a buffer solution containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

The cells are then lysed by passing the solution through a microfluidizer (Microfluidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then
5 mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 xg centrifugation for 15 min., the pellet is
10 discarded and the polypeptide containing supernatant is incubated at 4°C overnight to allow further GuHCl extraction.

Following high speed centrifugation (30,000 xg) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous
15 stirring. The refolded diluted protein solution is kept at 4°C without mixing for 12 hours prior to further purification steps.

To clarify the refolded polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16 µm membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is
20 loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

25 Fractions containing the polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM
30 NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH

6.5. Fractions are collected under constant A_{280} monitoring of the effluent. Fractions containing the polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant polypeptide should exhibit greater than 95% purity after the above refolding and purification steps. No major contaminant bands should be observed from
5 Commassie blue stained 16% SDS-PAGE gel when 5 μ g of purified protein is loaded. The purified protein can also be tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

Example 7: Cloning and Expression of a Polypeptide in a Baculovirus Expression System

10

In this example, the plasmid shuttle vector pA2 is used to insert a polynucleotide into a baculovirus to express a polypeptide. This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by
15 convenient restriction sites such as BamHI, Xba I and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak *Drosophila* promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides
20 by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned polynucleotide.

Many other baculovirus vectors can be used in place of the vector above, such as pAc373, pVL941, and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion
25 and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., *Virology* 170:31-39 (1989).

Specifically, the cDNA sequence contained in the deposited clone, including the AUG initiation codon, is amplified using the PCR protocol described in Example 1. If a naturally occurring signal sequence is used to produce a colon or colon cancer related
30 polypeptide, the pA2 vector does not need a second signal peptide. Alternatively, the vector can be modified (pA2 GP) to include a baculovirus leader sequence, using the standard methods described in Summers et al., "A Manual of Methods for Baculovirus Vectors and

Insect Cell Culture Procedures," Texas Agricultural Experimental Station Bulletin No. 1555 (1987).

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with
5 appropriate restriction enzymes and again purified on a 1% agarose gel.

The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("GeneClean" BIO 101 Inc., La Jolla, Ca.).

10 The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria containing the plasmid are identified by digesting DNA from individual colonies and analyzing the digestion product by gel electrophoresis. The sequence of the
15 cloned fragment is confirmed by DNA sequencing.

Five µg of a plasmid containing the polynucleotide is co-transfected with 1.0 µg of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417 (1987). One µg of BaculoGold™ virus DNA and 5 µg
20 of the plasmid are mixed in a sterile well of a microtiter plate containing 50 µl of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 µl Lipofectin plus 90 µl Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum.
25 The plate is then incubated for 5 hours at 27° C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27° C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, *supra*. An agarose gel with "Blue Gal" (Life Technologies
30 Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by

Life Technologies Inc., Gaithersburg, page 9- 10.) After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 μ l of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4° C.

To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus containing the polynucleotide at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5 μ Ci of 35 S-methionine and 5 μ Ci 35 S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the produced protein.

Example 8: Expression of a Polypeptide in Mammalian Cells

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The polypeptide of the present invention can be expressed in a mammalian cell. A typical mammalian expression vector contains a promoter element, which mediates the initiation of transcription of mRNA, a protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLV1, HIV1 and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter).

30

Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12MI (ATCC 67109), pCMVSPORT 2.0, and

pCMVSPORT 3.0. Mammalian host cells that could be used include, human HeLa, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the polypeptide can be expressed in stable cell lines containing the polynucleotide integrated into a chromosome. The co-transfection with a selectable marker such as DHFR, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that carry several hundred or even several thousand copies of the gene of interest. (See, e.g., Alt, F. W., et al., J. Biol. Chem. 253:1357-1370 (1978); Hamlin, J. L. and Ma, C., Biochem. et Biophys. Acta, 1097:107-143 (1990); Page, M. J. and Sydenham, M. A., Biotechnology 9:64-68 (1991).) Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., Biochem J. 227:277-279 (1991); Bebbington et al., Bio/Technology 10:169-175 (1992). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

Derivatives of the plasmid pSV2-dhfr (ATCC Accession No. 37146), the expression vectors pC4 (ATCC Accession No. 209646) and pC6 (ATCC Accession No. 209647) contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., Molecular and Cellular Biology, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart et al., Cell 41:521-530 (1985).) Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors also contain the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control of the SV40 early promoter.

Specifically, the plasmid pC6, for example, is digested with appropriate restriction enzymes and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

A polynucleotide of the present invention is amplified according to the protocol outlined in Example 1. If a naturally occurring signal sequence is used to produce the colon or colon cancer related polypeptide, the vector does not need a second signal peptide.

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Alternatively, if a naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The amplified fragment is then digested with the same restriction enzyme and purified on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC6 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene is used for transfection. Five μ g of the expression plasmid pC6 or pC4 is cotransfected with 0.5 μ g of the plasmid pSVneo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 μ M, 2 μ M, 5 μ M, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200 μ M. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

Example 9: Protein Fusions

The polypeptides of the present invention are preferably fused to other proteins. These fusion proteins can be used for a variety of applications. For example, fusion of the present polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification. (See Example 5; see also EP A 394,827; Traunecker, et al.,

Nature 331:84-86 (1988).) Similarly, fusion to IgG-1, IgG-3, and albumin increases the halflife time in vivo. Nuclear localization signals fused to the polypeptides of the present invention can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion proteins described above can be made by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule, or the protocol described in Example 5.

10 Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

For example, if pC4 (Accession No. 209646) is used, the human Fc portion can be 15 ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the vector, and a polynucleotide of the present invention, isolated by the PCR protocol described in Example 1, is ligated into this BamHI site. Note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be produced.

20 If the naturally occurring signal sequence is used to produce the colon or colon cancer related polypeptide, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

25 Human IgG Fc region:

GGGATCCGGAGCCCAAATCTTCTGACAAACTCACACATGCCCACCGTGCCCAG
CACCTGAATTCGAGGGTGCACCGTCAGTCTTCCTCTTCCCCCAAACCCAAGGA
CACCTCATGATCTCCCGGACTCCTGAGGTCACATGCGTGGTGGTGGACGTAAGC
CACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCAT
30 AATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC
AGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC
AAGGTCTCCAACAAAGCCCTCCCAACCCCATCGAGAAAACCATCTCCAAAGCC

AAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAG
CTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGC
GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGAC
CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACC
5 GTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCAT
GAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAT
GAGTGCGACGGCCGCGACTCTAGAGGAT (SEQ ID NO:8555)

Example 10: Production of an Antibody from a Polypeptide

10

a) Hybridoma Technology

The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing polypeptide of the present invention are administered to an animal to induce the production
15 of sera containing polyclonal antibodies. In a preferred method, a preparation of polypeptide of the present invention is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

Monoclonal antibodies specific for polypeptide of the present invention are prepared
20 using hybridoma technology. (Kohler et al., Nature 256:495 (1975); Kohler et al., Eur. J. Immunol. 6:511 (1976); Kohler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981)). In general, an animal (preferably a mouse) is immunized with polypeptide of the present invention or, more preferably, with a secreted polypeptide of the present invention-
25 expressing cell. Such polypeptide-expressing cells are cultured in any suitable tissue culture medium, preferably in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56°C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µg/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell
30 line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available

from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide of the present invention.

Alternatively, additional antibodies capable of binding to polypeptide of the present invention can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the polypeptide of the present invention-specific antibody can be blocked by polypeptide of the present invention. Such antibodies comprise anti-idiotypic antibodies to the polypeptide of the present invention-specific antibody and are used to immunize an animal to induce formation of further polypeptide of the present invention-specific antibodies.

For in vivo use of antibodies in humans, an antibody is "humanized". Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric and humanized antibodies are known in the art and are discussed herein. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

b) Isolation Of Antibody Fragments Directed Against Polypeptide of the Present Invention From A Library Of scFvs

Naturally occurring V-genes isolated from human PBLs are constructed into a library of antibody fragments which contain reactivities against polypeptide of the present invention to which the donor may or may not have been exposed (see e.g., U.S. Patent 5,885,793 incorporated herein by reference in its entirety).

Rescue of the Library. A library of scFvs is constructed from the RNA of human

PBLs as described in PCT publication WO 92/01047. To rescue phage displaying antibody fragments, approximately 10⁹ E. coli harboring the phagemid are used to inoculate 50 ml of 2xTY containing 1% glucose and 100 µg/ml of ampicillin (2xTY-AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to inoculate 50 ml of 2xTY-AMP-GLU, 2 x 10⁸ TU of delta gene 3 helper (M13 delta gene III, see PCT publication WO 92/01047) are added and the culture incubated at 37°C for 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 min. and the pellet resuspended in 2 liters of 2xTY containing 100 µg/ml ampicillin and 50 µg/ml kanamycin and grown overnight. Phage are prepared as described in PCT publication WO 92/01047.

M13 delta gene III is prepared as follows: M13 delta gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody fragments have a greater avidity of binding to antigen. Infectious M13 delta gene III particles are made by growing the helper phage in cells harboring a pUC19 derivative supplying the wild type gene III protein during phage morphogenesis. The culture is incubated for 1 hour at 37° C without shaking and then for a further hour at 37°C with shaking. Cells are spun down (IEC-Centra 8,400 r.p.m. for 10 min), resuspended in 300 ml 2xTY broth containing 100 µg ampicillin/ml and 25 µg kanamycin/ml (2xTY-AMP-KAN) and grown overnight, shaking at 37°C. Phage particles are purified and concentrated from the culture medium by two PEG-precipitations (Sambrook et al., 1990), resuspended in 2 ml PBS and passed through a 0.45 µm filter (Minisart NML; Sartorius) to give a final concentration of approximately 10¹³ transducing units/ml (ampicillin-resistant clones).

Panning of the Library. Immunotubes (Nunc) are coated overnight in PBS with 4 ml of either 100 µg/ml or 10 µg/ml of a polypeptide of the present invention. Tubes are blocked with 2% Marvel-PBS for 2 hours at 37°C and then washed 3 times in PBS. Approximately 10¹³ TU of phage is applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml of mid-log E. coli TG1 by incubating eluted phage with bacteria for 30 minutes at 37°C. The E. coli are then plated on TYE plates containing 1%

glucose and 100 µg/ml ampicillin. The resulting bacterial library is then rescued with delta gene 3 helper phage as described above to prepare phage for a subsequent round of selection. This process is then repeated for a total of 4 rounds of affinity purification with tube-washing increased to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

Characterization of Binders. Eluted phage from the 3rd and 4th rounds of selection are used to infect E. coli HB 2151 and soluble scFv is produced (Marks, et al., 1991) from single colonies for assay. ELISAs are performed with microtitre plates coated with either 10 pg/ml of the polypeptide of the present invention in 50 mM bicarbonate pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see, e.g., PCT publication WO 92/01047) and then by sequencing. These ELISA positive clones may also be further characterized by techniques known in the art, such as, for example, epitope mapping, binding affinity, receptor signal transduction, ability to block or competitively inhibit antibody/antigen binding, and competitive agonistic or antagonistic activity.

15

Example 11: Method of Determining Alterations in a Gene Corresponding to a Polynucleotide

RNA isolated from entire families or individual patients presenting with a phenotype of interest (such as a disease) is be isolated. cDNA is then generated from these RNA samples using protocols known in the art. (See, Sambrook.) The cDNA is then used as a template for PCR, employing primers surrounding regions of interest in SEQ ID NO:X. Suggested PCR conditions consist of 35 cycles at 95 degrees C for 30 seconds; 60-120 seconds at 52-58 degrees C; and 60-120 seconds at 70 degrees C, using buffer solutions described in Sidransky et al., Science 252:706 (1991).

PCR products are then sequenced using primers labeled at their 5' end with T4 polynucleotide kinase, employing SequiTherm Polymerase. (Epicentre Technologies). The intron-exon borders of selected exons is also determined and genomic PCR products analyzed to confirm the results. PCR products harboring suspected mutations is then cloned and sequenced to validate the results of the direct sequencing.

30

PCR products is cloned into T-tailed vectors as described in Holton et al., Nucleic Acids Research, 19:1156 (1991) and sequenced with T7 polymerase (United States

Biochemical). Affected individuals are identified by mutations not present in unaffected individuals.

Genomic rearrangements are also observed as a method of determining alterations in a gene corresponding to a polynucleotide. Genomic clones isolated according to Example 2
5 are nick-translated with digoxigenin deoxy-uridine 5'-triphosphate (Boehringer Mannheim), and FISH performed as described in Johnson et al., Methods Cell Biol. 35:73-99 (1991). Hybridization with the labeled probe is carried out using a vast excess of human cot-1 DNA for specific hybridization to the corresponding genomic locus.

Chromosomes are counterstained with 4,6-diamino-2-phenylidole and propidium
10 iodide, producing a combination of C- and R-bands. Aligned images for precise mapping are obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination with a cooled charge-coupled device camera (Photometrics, Tucson, AZ) and variable excitation wavelength filters. (Johnson et al., Genet. Anal. Tech. Appl., 8:75 (1991).) Image collection, analysis and chromosomal fractional length measurements are performed using
15 the ISee Graphical Program System. (Inovision Corporation, Durham, NC.) Chromosome alterations of the genomic region hybridized by the probe are identified as insertions, deletions, and translocations. These alterations are used as a diagnostic marker for an associated disease.

20 ***Example 12: Method of Detecting Abnormal Levels of a Polypeptide in a Biological Sample***

A polypeptide of the present invention can be detected in a biological sample, and if an increased or decreased level of the polypeptide is detected, this polypeptide is a marker for
25 a particular phenotype. Methods of detection are numerous, and thus, it is understood that one skilled in the art can modify the following assay to fit their particular needs.

For example, antibody-sandwich ELISAs are used to detect polypeptides in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies, at a final concentration of 0.2 to 10 ug/ml. The antibodies are either monoclonal
30 or polyclonal and are produced by the method described in Example 10. The wells are blocked so that non-specific binding of the polypeptide to the well is reduced.

The coated wells are then incubated for > 2 hours at RT with a sample containing the polypeptide. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove unbound polypeptide.

5 Next, 50 ul of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature. The plates are again washed three times with deionized or distilled water to remove unbounded conjugate.

Add 75 μ l of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard curve, using serial dilutions of a control sample, and plot polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale). Interpolate the concentration of the polypeptide in the sample using the standard curve.

15 **Example 13: Formulation**

The invention also provides methods of treatment and/or prevention of diseases or disorders (such as, for example, any one or more of the diseases or disorders disclosed herein) by administration to a subject of an effective amount of a Therapeutic. By
20 therapeutic is meant a polynucleotides or polypeptides of the invention (including fragments and variants), agonists or antagonists thereof, and/or antibodies thereto, in combination with a pharmaceutically acceptable carrier type (e.g., a sterile carrier).

The Therapeutic will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the Therapeutic alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of the Therapeutic administered parenterally per dose will be in the range of about 1ug/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given

continuously, the Therapeutic is typically administered at a dose rate of about 1 ug/kg/hour to about 50 ug/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Therapeutics can be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics are administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics include suitable polymeric materials (such as, for example, semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules), suitable hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman et al., Biopolymers 22:547-556 (1983)), poly (2-hydroxyethyl methacrylate) (Langer et al., J. Biomed. Mater. Res. 15:167-277 (1981), and Langer, Chem. Tech. 12:98-105 (1982)), ethylene vinyl acetate (Langer et al., Id.) or poly-D-(-)-3-hydroxybutyric acid (EP 133,988).

Sustained-release Therapeutics also include liposomally entrapped Therapeutics of the invention (see generally, Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317 -327 and 353-365 (1989)). Liposomes containing the
5 Therapeutic are prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci.(USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid
10 content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal Therapeutic.

In yet an additional embodiment, the Therapeutics of the invention are delivered by way of a pump (see Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)).

15 Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

For parenteral administration, in one embodiment, the Therapeutic is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that
20 is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to the Therapeutic.

Generally, the formulations are prepared by contacting the Therapeutic uniformly and
25 intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

30 The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate,

succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The Therapeutic is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

Any pharmaceutical used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutics generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Therapeutics ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous Therapeutic solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized Therapeutic using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the Therapeutics of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the Therapeutics may be employed in conjunction with other therapeutic compounds.

The Therapeutics of the invention may be administered alone or in combination with adjuvants. Adjuvants that may be administered with the Therapeutics of the invention

include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG, and MPL. In a specific embodiment, Therapeutics of the invention are administered in combination with alum. In another specific embodiment, Therapeutics of the invention are administered in combination with QS-21.

5 Further adjuvants that may be administered with the Therapeutics of the invention include, but are not limited to, Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59, and Virosomal adjuvant technology. Vaccines that may be administered with the Therapeutics of the invention include, but are not limited to, vaccines directed toward protection against MMR (measles, mumps, rubella), polio,

10 varicella, tetanus/diphtheria, hepatitis A, hepatitis B, haemophilus influenzae B, whooping cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are

15 administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

The Therapeutics of the invention may be administered alone or in combination with

20 other therapeutic agents. Therapeutic agents that may be administered in combination with the Therapeutics of the invention, include but not limited to, other members of the TNF family, chemotherapeutic agents, antibiotics, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents, cytokines and/or growth factors. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or

25 concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

30 In one embodiment, the Therapeutics of the invention are administered in combination with members of the TNF family. TNF, TNF-related or TNF-like molecules that may be administered with the Therapeutics of the invention include, but are not limited

to, soluble forms of TNF-alpha, lymphotoxin- alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), endokine-alpha
5 (International Publication No. WO 98/07880), TR6 (International Publication No. WO 98/30694), OPG, and neutrokin-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International
10 Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TR7 (International Publication No. WO 98/41629), TRANK, TR9 (International Publication No. WO 98/56892), TR10 (International Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153.

In certain embodiments, Therapeutics of the invention are administered in combination with
15 antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors. Nucleoside reverse transcriptase inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and
20 COMBIVIR™ (zidovudine/lamivudine). Non-nucleoside reverse transcriptase inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, VIRAMUNE™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, CRIVIVAN™ (indinavir),
25 NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with Therapeutics of the invention to treat AIDS and/or to prevent or treat HIV infection.

30 In other embodiments, Therapeutics of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the Therapeutics of the invention, include, but are not

limited to, TRIMETHOPRIM- SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™, CLARITHROMYCIN™, AZITHROMYCIN™, GANCICLOVIR™, FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, 5 ITRACONAZOLE™, KETOCONAZOLE™, ACYCLOVIR™, FAMCICOLVIR™, PYRIMETHAMINE™, LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and LEUKINE™ (sargramostim/GM-CSF). In a specific embodiment, Therapeutics of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, and/or ATOVAQUONE™ to prophylactically treat or 10 prevent an opportunistic *Pneumocystis carinii* pneumonia infection. In another specific embodiment, Therapeutics of the invention are used in any combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or ETHAMBUTOL™ to prophylactically treat or prevent an opportunistic *Mycobacterium avium* complex infection. In another specific embodiment, Therapeutics of the invention are used in any combination with RIFABUTIN™, 15 CLARITHROMYCIN™, and/or AZITHROMYCIN™ to prophylactically treat or prevent an opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment, Therapeutics of the invention are used in any combination with GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat or prevent an opportunistic cytomegalovirus infection. In another specific embodiment, Therapeutics of the invention are used in any combination with FLUCONAZOLE™, ITRACONAZOLE™, and/or 20 KETOCONAZOLE™ to prophylactically treat or prevent an opportunistic fungal infection. In another specific embodiment, Therapeutics of the invention are used in any combination with ACYCLOVIR™ and/or FAMCICOLVIR™ to prophylactically treat or prevent an opportunistic herpes simplex virus type I and/or type II infection. In another specific 25 embodiment, Therapeutics of the invention are used in any combination with PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylactically treat or prevent an opportunistic *Toxoplasma gondii* infection. In another specific embodiment, Therapeutics of the invention are used in any combination with LEUCOVORIN™ and/or NEUPOGEN™ to prophylactically treat or prevent an opportunistic bacterial infection.

30 In a further embodiment, the Therapeutics of the invention are administered in combination with an antiviral agent. Antiviral agents that may be administered with the

Therapeutics of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.

In a further embodiment, the Therapeutics of the invention are administered in combination with an antibiotic agent. Antibiotic agents that may be administered with the
5 Therapeutics of the invention include, but are not limited to, amoxicillin, beta-lactamases, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin, chloramphenicol, cephalosporins, ciprofloxacin, ciprofloxacin, erythromycin, fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamthoxazole, and vancomycin.

10 Conventional nonspecific immunosuppressive agents, that may be administered in combination with the Therapeutics of the invention include, but are not limited to, steroids, cyclosporine, cyclosporine analogs, cyclophosphamide methylprednisone, prednisone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T cells.

15 In specific embodiments, Therapeutics of the invention are administered in combination with immunosuppressants. Immunosuppressants preparations that may be administered with the Therapeutics of the invention include, but are not limited to, ORTHOCLONE™ (OKT3), SANDIMMUNE™/NEORAL™/SANGDYA™ (cyclosporin), PROGRAF™ (tacrolimus), CELLCEPT™ (mycophenolate), Azathioprine, glucorticosteroids,
20 and RAPAMUNE™ (sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

In an additional embodiment, Therapeutics of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the Therapeutics of the
25 invention include, but not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, and GAMIMUNE™. In a specific embodiment, Therapeutics of the invention are administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

In an additional embodiment, the Therapeutics of the invention are administered alone
30 or in combination with an anti-inflammatory agent. Anti-inflammatory agents that may be administered with the Therapeutics of the invention include, but are not limited to, glucocorticoids and the nonsteroidal anti-inflammatories, aminoarylcarboxylic acid

derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, 5 emorfazone, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranyline, perisoxal, pifoxime, proquazone, proxazole, and tenidap.

In another embodiment, compositions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the Therapeutics of the invention include, but are not limited to, antibiotic 10 derivatives (e.g., doxorubicin, bleomycin, daunorubicin, and dactinomycin); antiestrogens (e.g., tamoxifen); antimetabolites (e.g., fluorouracil, 5-FU, methotrexate, floxuridine, interferon alpha-2b, glutamic acid, plicamycin, mercaptopurine, and 6-thioguanine); cytotoxic agents (e.g., carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin, busulfan, cis-platin, 15 and vincristine sulfate); hormones (e.g., medroxyprogesterone, estramustine phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone); nitrogen mustard derivatives (e.g., mephallen, chorambucil, mechlorethamine (nitrogen mustard) and thiotepa); steroids and combinations (e.g., bethamethasone sodium phosphate); and others (e.g., dicarbazine, 20 asparaginase, mitotane, vincristine sulfate, vinblastine sulfate, and etoposide).

In a specific embodiment, Therapeutics of the invention are administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or any combination of the components of CHOP. In another embodiment, Therapeutics of the invention are administered in combination with Rituximab. In a further embodiment, 25 Therapeutics of the invention are administered with Rituxmab and CHOP, or Rituxmab and any combination of the components of CHOP.

In an additional embodiment, the Therapeutics of the invention are administered in combination with cytokines. Cytokines that may be administered with the Therapeutics of the invention include, but are not limited to, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, 30 IL15, anti-CD40, CD40L, IFN-gamma and TNF-alpha. In another embodiment, Therapeutics of the invention may be administered with any interleukin, including, but not

limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL- 4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, and IL-21.

In an additional embodiment, the Therapeutics of the invention are administered in combination with angiogenic proteins. Angiogenic proteins that may be administered with the Therapeutics of the invention include, but are not limited to, Glioma Derived Growth Factor (GDGF), as disclosed in European Patent Number EP-399816; Platelet Derived Growth Factor-A (PDGF-A), as disclosed in European Patent Number EP-682110; Platelet Derived Growth Factor-B (PDGF-B), as disclosed in European Patent Number EP-282317; Placental Growth Factor (PIGF), as disclosed in International Publication Number WO 92/06194; Placental Growth Factor-2 (PIGF-2), as disclosed in Hauser et al., Growth Factors, 4:259-268 (1993); Vascular Endothelial Growth Factor (VEGF), as disclosed in International Publication Number WO 90/13649; Vascular Endothelial Growth Factor-A (VEGF-A), as disclosed in European Patent Number EP-506477; Vascular Endothelial Growth Factor-2 (VEGF-2), as disclosed in International Publication Number WO 96/39515; Vascular Endothelial Growth Factor B (VEGF-3); Vascular Endothelial Growth Factor B-186 (VEGF-B186), as disclosed in International Publication Number WO 96/26736; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/02543; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/07832; and Vascular Endothelial Growth Factor-E (VEGF-E), as disclosed in German Patent Number DE19639601. The above mentioned references are incorporated herein by reference herein.

In an additional embodiment, the Therapeutics of the invention are administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the Therapeutics of the invention include, but are not limited to, LEUKINE™ (SARGRAMOSTIM™) and NEUPOGEN™ (FILGRASTIM™).

In an additional embodiment, the Therapeutics of the invention are administered in combination with Fibroblast Growth Factors. Fibroblast Growth Factors that may be administered with the Therapeutics of the invention include, but are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

In additional embodiments, the Therapeutics of the invention are administered in combination with other therapeutic or prophylactic regimens, such as, for example, radiation therapy.

5 ***Example 14: Method of Treating Decreased Levels of the Polypeptide***

The present invention relates to a method for treating an individual in need of an increased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an agonist
10 of the invention (including polypeptides of the invention). Moreover, it will be appreciated that conditions caused by a decrease in the standard or normal expression level of a colon or colon cancer related polypeptide in an individual can be treated by administering the agonist or antagonist of the present invention. Thus, the invention also provides a method of treatment of an individual in need of an increased level of the polypeptide comprising
15 administering to such an individual a Therapeutic comprising an amount of the agonist or antagonist to increase the activity level of the polypeptide in such an individual.

For example, a patient with decreased levels of a polypeptide receives a daily dose 0.1-100 ug/kg of the agonist or antagonist for six consecutive days. The exact details of the dosing scheme, based on administration and formulation, are provided in Example 13.

20

Example 15: Method of Treating Increased Levels of the Polypeptide

The present invention also relates to a method of treating an individual in need of a decreased level of a polypeptide of the invention in the body comprising administering to
25 such an individual a composition comprising a therapeutically effective amount of an antagonist of the invention (including polypeptides and antibodies of the invention).

In one example, antisense technology is used to inhibit production of a polypeptide of the present invention. This technology is one example of a method of decreasing levels of a polypeptide, due to a variety of etiologies, such as cancer.

30 For example, a patient diagnosed with abnormally increased levels of a polypeptide is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day

for 21 days. This treatment is repeated after a 7-day rest period if the treatment was well tolerated. The formulation of the antisense polynucleotide is provided in Example 13.

Example 16: Method of Treatment Using Gene Therapy-Ex Vivo

5

One method of gene therapy transplants fibroblasts, which are capable of expressing a polypeptide, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately
10 ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37 degree C for approximately one week.

15 At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks.

pMV-7 (Kirschmeier, P.T. et al., DNA, 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII
20 and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

The cDNA encoding a polypeptide of the present invention can be amplified using PCR primers which correspond to the 5' and 3' end sequences respectively as set forth in Example 1 using primers and having appropriate restriction sites and initiation/stop codons, if
25 necessary. Preferably, the 5' primer contains an EcoRI site and the 3' primer includes a HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform bacteria HB101, which are then
30 plated onto agar containing kanamycin for the purpose of confirming that the vector has the gene of interest properly inserted.

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The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the gene is then added to the media and the packaging cells transduced with the vector. The packaging cells now produce
5 infectious viral particles containing the gene (the packaging cells are now referred to as producer cells).

Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer
10 cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his.
15 Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether protein is produced.

The engineered fibroblasts are then transplanted onto the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads.

20 ***Example 17: Gene Therapy Using Endogenous Genes Corresponding To Polynucleotides of the Invention***

Another method of gene therapy according to the present invention involves operably associating the endogenous polynucleotide sequence of the invention with a promoter via
25 homologous recombination as described, for example, in U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., *Proc. Natl. Acad. Sci. USA*, 86:8932-8935 (1989); and Zijlstra et al., *Nature*, 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is
30 not expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made which contain a promoter and targeting sequences, which are homologous to the 5' non-coding sequence of endogenous

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polynucleotide sequence, flanking the promoter. The targeting sequence will be sufficiently near the 5' end of the polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination. The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains
5 distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter.

The amplified promoter and the amplified targeting sequences are digested with the
10 appropriate restriction enzymes and subsequently treated with calf intestinal phosphatase. The digested promoter and digested targeting sequences are added together in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The construct is size fractionated on an agarose gel then purified by phenol extraction and ethanol precipitation.

15 In this Example, the polynucleotide constructs are administered as naked polynucleotides via electroporation. However, the polynucleotide constructs may also be administered with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, precipitating agents, etc. Such methods of delivery are known in the art.

Once the cells are transfected, homologous recombination will take place which
20 results in the promoter being operably linked to the endogenous polynucleotide sequence. This results in the expression of polynucleotide corresponding to the polynucleotide in the cell. Expression may be detected by immunological staining, or any other method known in the art.

Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed
25 in DMEM + 10% fetal calf serum. Exponentially growing or early stationary phase fibroblasts are trypsinized and rinsed from the plastic surface with nutrient medium. An aliquot of the cell suspension is removed for counting, and the remaining cells are subjected to centrifugation. The supernatant is aspirated and the pellet is resuspended in 5 ml of electroporation buffer (20 mM HEPES pH 7.3, 137 mM NaCl, 5 mM KCl, 0.7 mM Na₂
30 HPO₄, 6 mM dextrose). The cells are recentrifuged, the supernatant aspirated, and the cells resuspended in electroporation buffer containing 1 mg/ml acetylated bovine serum albumin.

The final cell suspension contains approximately 3×10^6 cells/ml. Electroporation should be performed immediately following resuspension.

Plasmid DNA is prepared according to standard techniques. For example, to construct a plasmid for targeting to the locus corresponding to the polynucleotide of the invention, plasmid pUC18 (MBI Fermentas, Amherst, NY) is digested with HindIII. The CMV promoter is amplified by PCR with an XbaI site on the 5' end and a BamHI site on the 3' end. Two non-coding sequences are amplified via PCR: one non-coding sequence (fragment 1) is amplified with a HindIII site at the 5' end and an Xba site at the 3' end; the other non-coding sequence (fragment 2) is amplified with a BamHI site at the 5' end and a HindIII site at the 3' end. The CMV promoter and the fragments (1 and 2) are digested with the appropriate enzymes (CMV promoter - XbaI and BamHI; fragment 1 - XbaI; fragment 2 - BamHI) and ligated together. The resulting ligation product is digested with HindIII, and ligated with the HindIII-digested pUC18 plasmid.

Plasmid DNA is added to a sterile cuvette with a 0.4 cm electrode gap (Bio-Rad). The final DNA concentration is generally at least 120 µg/ml. 0.5 ml of the cell suspension (containing approximately 1.5×10^6 cells) is then added to the cuvette, and the cell suspension and DNA solutions are gently mixed. Electroporation is performed with a Gene-Pulser apparatus (Bio-Rad). Capacitance and voltage are set at 960 µF and 250-300 V, respectively. As voltage increases, cell survival decreases, but the percentage of surviving cells that stably incorporate the introduced DNA into their genome increases dramatically. Given these parameters, a pulse time of approximately 14-20 mSec should be observed.

Electroporated cells are maintained at room temperature for approximately 5 min, and the contents of the cuvette are then gently removed with a sterile transfer pipette. The cells are added directly to 10 ml of prewarmed nutrient media (DMEM with 15% calf serum) in a 10 cm dish and incubated at 37 degree C. The following day, the media is aspirated and replaced with 10 ml of fresh media and incubated for a further 16-24 hours.

The engineered fibroblasts are then injected into the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads. The fibroblasts now produce the protein product. The fibroblasts can then be introduced into a patient as described above.

Example 18: Method of Treatment Using Gene Therapy - In Vivo

Another aspect of the present invention is using *in vivo* gene therapy methods to treat disorders, diseases and conditions. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) sequences into an animal to increase or decrease the expression of the polypeptide. The polynucleotide of the present invention may be operatively linked to a promoter or any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques and methods are known in the art, see, for example, WO90/11092, WO98/11779; U.S. Patent NO. 5693622, 5705151, 5580859; Tabata et al., Cardiovasc. Res. 35(3):470-479 (1997); Chao et al., Pharmacol. Res. 35(6):517-522 (1997); Wolff, Neuromuscul. Disord. 7(5):314-318 (1997); Schwartz et al., Gene Ther. 3(5):405-411 (1996); Tsurumi et al., Circulation 94(12):3281-3290 (1996) (incorporated herein by reference).

The polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, intestine and the like). The polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to assist, promote, or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotides of the present invention may also be delivered in liposome formulations (such as those taught in Felgner P.L. et al. (1995) Ann. NY Acad. Sci. 772:126-139 and Abdallah B. et al. (1995) Biol. Cell 85(1):1-7) which can be prepared by methods well known to those skilled in the art.

The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in the art can be used for driving the expression of DNA. Unlike other gene therapies techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus,

heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 g/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The dose response effects of injected polynucleotide in muscle *in vivo* is determined as follows. Suitable template DNA for production of mRNA coding for polypeptide of the present invention is prepared in accordance with a standard recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps muscles of mice are then injected with various amounts of the template DNA.

Five to six week old female and male Balb/C mice are anesthetized by intraperitoneal

injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on the anterior thigh, and the quadriceps muscle is directly visualized. The template DNA is injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over one minute, approximately 0.5 cm from the distal insertion site of the muscle into the knee and about 0.2 cm deep. A suture is placed over the injection site for future localization, and the skin is closed with stainless steel clips.

After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15 um cross-section of the individual quadriceps muscles is histochemically stained for protein expression. A time course for protein expression may be done in a similar fashion except that quadriceps from different mice are harvested at different times. Persistence of DNA in muscle following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice. The results of the above experimentation in mice can be used to extrapolate proper dosages and other treatment parameters in humans and other animals using naked DNA.

Example 19: Transgenic Animals

The polypeptides of the invention can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters, guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, e.g., baboons, monkeys, and chimpanzees may be used to generate transgenic animals. In a specific embodiment, techniques described herein or otherwise known in the art, are used to express polypeptides of the invention in humans, as part of a gene therapy protocol.

Any technique known in the art may be used to introduce the transgene (i.e., polynucleotides of the invention) into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (Paterson et al., Appl. Microbiol. Biotechnol. 40:691-698 (1994); Carver et al., Biotechnology (NY) 11:1263-1270 (1993); Wright et al., Biotechnology (NY) 9:830-834 (1991); and Hoppe et al., U.S. Pat. No. 4,873,191 (1989)); retrovirus mediated gene transfer into germ lines (Van der Putten et al., Proc. Natl. Acad. Sci., USA 82:6148-6152 (1985)), blastocysts or embryos; gene targeting in embryonic stem cells (Thompson et al., Cell 56:313-321 (1989));

electroporation of cells or embryos (Lo, 1983, Mol Cell Biol. 3:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (see, e.g., Ulmer et al., Science 259:1745 (1993); introducing nucleic acid constructs into embryonic pluripotent stem cells and transferring the stem cells back into the blastocyst; and sperm-mediated gene transfer (Lavitrano et al., Cell 57:717-723 (1989); etc. For a review of such techniques, see
5 Gordon, "Transgenic Animals," Intl. Rev. Cytol. 115:171-229 (1989), which is incorporated by reference herein in its entirety.

Any technique known in the art may be used to produce transgenic clones containing polynucleotides of the invention, for example, nuclear transfer into enucleated oocytes of
10 nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (Campell et al., Nature 380:64-66 (1996); Wilmut et al., Nature 385:810-813 (1997)).

The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, *i.e.*, mosaic animals or chimeric. The transgene may be integrated as a single transgene or as
15 multiple copies such as in concatamers, *e.g.*, head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko et al., Proc. Natl. Acad. Sci. USA 89:6232-6236 (1992)). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those
20 of skill in the art. When it is desired that the polynucleotide transgene be integrated into the chromosomal site of the endogenous gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of
25 the endogenous gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type, by following, for example, the teaching of Gu et al. (Gu et al., Science 265:103-106 (1994)). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

30 Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration

of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and reverse transcriptase-PCR (rt-PCR). Samples of transgenic gene-expressing tissue may also be evaluated immunocytochemically or immunohistochemically using antibodies specific for the transgene product.

Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher levels because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; and breeding to place the transgene on a distinct background that is appropriate for an experimental model of interest.

Transgenic animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying conditions and/or disorders associated with aberrant expression, and in screening for compounds effective in ameliorating such conditions and/or disorders.

Example 20: Knock-Out Animals

Endogenous gene expression can also be reduced by inactivating or "knocking out" the gene and/or its promoter using targeted homologous recombination. (*E.g.*, see Smithies et al., *Nature* 317:230-234 (1985); Thomas & Capecchi, *Cell* 51:503-512 (1987); Thompson et al., *Cell* 5:313-321 (1989); each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional polynucleotide of the invention (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous polynucleotide sequence (either the coding regions or regulatory regions of the gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect

cells that express polypeptides of the invention *in vivo*. In another embodiment, techniques known in the art are used to generate knockouts in cells that contain, but do not express the gene of interest. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the targeted gene. Such approaches are particularly suited in research and agricultural fields where modifications to embryonic stem cells can be used to generate animal offspring with an inactive targeted gene (*e.g.*, see Thomas & Capecchi 1987 and Thompson 1989, *supra*). However this approach can be routinely adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors that will be apparent to those of skill in the art.

In further embodiments of the invention, cells that are genetically engineered to express the polypeptides of the invention, or alternatively, that are genetically engineered not to express the polypeptides of the invention (*e.g.*, knockouts) are administered to a patient *in vivo*. Such cells may be obtained from the patient (*i.e.*, animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (*e.g.*, lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention, *e.g.*, by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc. The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, *e.g.*, in the circulation, or intraperitoneally.

Alternatively, the cells can be incorporated into a matrix and implanted in the body, *e.g.*, genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U.S. Patent No. 5,399,349; and Mulligan & Wilson, U.S. Patent No. 5,460,959 each of which is incorporated by reference herein in its entirety).

When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

Transgenic and "knock-out" animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying conditions and/or disorders associated with aberrant expression, and in screening for compounds effective in ameliorating such conditions and/or disorders.

***Example 21: Assays Detecting Stimulation or Inhibition of B cell
Proliferation and Differentiation***

Generation of functional humoral immune responses requires both soluble and cognate signaling between B-lineage cells and their microenvironment. Signals may impart a positive stimulus that allows a B-lineage cell to continue its programmed development, or a negative stimulus that instructs the cell to arrest its current developmental pathway. To date, numerous stimulatory and inhibitory signals have been found to influence B cell responsiveness including IL-2, IL-4, IL-5, IL-6, IL-7, IL10, IL-13, IL-14 and IL-15. Interestingly, these signals are by themselves weak effectors but can, in combination with various co-stimulatory proteins, induce activation, proliferation, differentiation, homing, tolerance and death among B cell populations.

One of the best studied classes of B-cell co-stimulatory proteins is the TNF-superfamily. Within this family CD40, CD27, and CD30 along with their respective ligands CD154, CD70, and CD153 have been found to regulate a variety of immune responses. Assays which allow for the detection and/or observation of the proliferation and differentiation of these B-cell populations and their precursors are valuable tools in determining the effects various proteins may have on these B-cell populations in terms of proliferation and differentiation. Listed below are two assays designed to allow for the

detection of the differentiation, proliferation, or inhibition of B-cell populations and their precursors.

In Vitro Assay- Agonists or antagonists of the invention can be assessed for its ability to induce activation, proliferation, differentiation or inhibition and/or death in B-cell populations and their precursors. The activity of the agonists or antagonists of the invention on purified human tonsillar B cells, measured qualitatively over the dose range from 0.1 to 10,000 ng/mL, is assessed in a standard B-lymphocyte co-stimulation assay in which purified tonsillar B cells are cultured in the presence of either formalin-fixed *Staphylococcus aureus* Cowan I (SAC) or immobilized anti-human IgM antibody as the priming agent. Second signals such as IL-2 and IL-15 synergize with SAC and IgM crosslinking to elicit B cell proliferation as measured by tritiated-thymidine incorporation. Novel synergizing agents can be readily identified using this assay. The assay involves isolating human tonsillar B cells by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population is greater than 95% B cells as assessed by expression of CD45R(B220).

Various dilutions of each sample are placed into individual wells of a 96-well plate to which are added 10^5 B-cells suspended in culture medium (RPMI 1640 containing 10% FBS, 5×10^{-5} M 2ME, 100U/ml penicillin, 10ug/ml streptomycin, and 10^{-5} dilution of SAC) in a total volume of 150ul. Proliferation or inhibition is quantitated by a 20h pulse (1uCi/well) with 3H-thymidine (6.7 Ci/mM) beginning 72h post factor addition. The positive and negative controls are IL2 and medium respectively.

In Vivo Assay- BALB/c mice are injected (i.p.) twice per day with buffer only, or 2 mg/Kg of agonists or antagonists of the invention, or truncated forms thereof. Mice receive this treatment for 4 consecutive days, at which time they are sacrificed and various tissues and serum collected for analyses. Comparison of H&E sections from normal spleens and spleens treated with agonists or antagonists of the invention identify the results of the activity of the agonists or antagonists on spleen cells, such as the diffusion of peri-arterial lymphatic sheaths, and/or significant increases in the nucleated cellularity of the red pulp regions, which may indicate the activation of the differentiation and proliferation of B-cell populations. Immunohistochemical studies using a B cell marker, anti-CD45R(B220), are used to determine whether any physiological changes to splenic cells, such as splenic disorganization, are due to increased B-cell representation within loosely defined B-cell zones that infiltrate established T-cell regions.

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Flow cytometric analyses of the spleens from mice treated with agonist or antagonist is used to indicate whether the agonists or antagonists specifically increases the proportion of ThB+, CD45R(B220)dull B cells over that which is observed in control mice.

5 Likewise, a predicted consequence of increased mature B-cell representation in vivo is a relative increase in serum Ig titers. Accordingly, serum IgM and IgA levels are compared between buffer and agonists or antagonists-treated mice.

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

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Example 22: T Cell Proliferation Assay

A CD3-induced proliferation assay is performed on PBMCs and is measured by the uptake of ³H-thymidine. The assay is performed as follows. Ninety-six well plates are
15 coated with 100 µl/well of mAb to CD3 (HIT3a, Pharmingen) or isotype-matched control mAb (B33.1) overnight at 4 degrees C (1 µg/ml in .05M bicarbonate buffer, pH 9.5), then washed three times with PBS. PBMC are isolated by F/H gradient centrifugation from human peripheral blood and added to quadruplicate wells (5 x 10⁴/well) of mAb coated plates in RPMI containing 10% FCS and P/S in the presence of varying concentrations of agonists
20 or antagonists of the invention (total volume 200 ul). Relevant protein buffer and medium alone are controls. After 48 hr. culture at 37 degrees C, plates are spun for 2 min. at 1000 rpm and 100 µl of supernatant is removed and stored -20 degrees C for measurement of IL-2 (or other cytokines) if effect on proliferation is observed. Wells are supplemented with 100 ul of medium containing 0.5 uCi of ³H-thymidine and cultured at 37 degrees C for 18-24 hr.
25 Wells are harvested and incorporation of ³H-thymidine used as a measure of proliferation. Anti-CD3 alone is the positive control for proliferation. IL-2 (100 U/ml) is also used as a control which enhances proliferation. Control antibody which does not induce proliferation of T cells is used as the negative controls for the effects of agonists or antagonists of the invention.

30 The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

Example 23: Effect of Agonists or Antagonists of the Invention on the Expression of MHC Class II, Costimulatory and Adhesion Molecules and Cell Differentiation of Monocytes and Monocyte-Derived Human Dendritic Cells

5

Dendritic cells are generated by the expansion of proliferating precursors found in the peripheral blood: adherent PBMC or elutriated monocytic fractions are cultured for 7-10 days with GM-CSF (50 ng/ml) and IL-4 (20 ng/ml). These dendritic cells have the characteristic
10 phenotype of immature cells (expression of CD1, CD80, CD86, CD40 and MHC class II antigens). Treatment with activating factors, such as TNF- α , causes a rapid change in surface phenotype (increased expression of MHC class I and II, costimulatory and adhesion molecules, downregulation of FC γ RII, upregulation of CD83). These changes correlate with increased antigen-presenting capacity and with functional maturation of the dendritic cells.

15 FACS analysis of surface antigens is performed as follows. Cells are treated 1-3 days with increasing concentrations of agonist or antagonist of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degrees C. After an additional wash, the labeled cells are analyzed by flow
20 cytometry on a FACScan (Becton Dickinson).

Effect on the production of cytokines. Cytokines generated by dendritic cells, in particular IL-12, are important in the initiation of T-cell dependent immune responses. IL-12 strongly influences the development of Th1 helper T-cell immune response, and induces cytotoxic T
25 and NK cell function. An ELISA is used to measure the IL-12 release as follows. Dendritic cells (10^6 /ml) are treated with increasing concentrations of agonists or antagonists of the invention for 24 hours. LPS (100 ng/ml) is added to the cell culture as positive control. Supernatants from the cell cultures are then collected and analyzed for IL-12 content using commercial ELISA kit (e.g., R & D Systems (Minneapolis, MN)). The standard protocols
30 provided with the kits are used.

Effect on the expression of MHC Class II, costimulatory and adhesion molecules. Three major families of cell surface antigens can be identified on monocytes: adhesion molecules, molecules involved in antigen presentation, and Fc receptor. Modulation of the expression of MHC class II antigens and other costimulatory molecules, such as B7 and ICAM-1, may result in changes in the antigen presenting capacity of monocytes and ability to induce T cell activation. Increase expression of Fc receptors may correlate with improved monocyte cytotoxic activity, cytokine release and phagocytosis.

FACS analysis is used to examine the surface antigens as follows. Monocytes are treated 1-5 days with increasing concentrations of agonists or antagonists of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degreesC. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

Monocyte activation and/or increased survival. Assays for molecules that activate (or alternatively, inactivate) monocytes and/or increase monocyte survival (or alternatively, decrease monocyte survival) are known in the art and may routinely be applied to determine whether a molecule of the invention functions as an inhibitor or activator of monocytes. Agonists or antagonists of the invention can be screened using the three assays described below. For each of these assays, Peripheral blood mononuclear cells (PBMC) are purified from single donor leukopacks (American Red Cross, Baltimore, MD) by centrifugation through a Histopaque gradient (Sigma). Monocytes are isolated from PBMC by counterflow centrifugal elutriation.

Monocyte Survival Assay. Human peripheral blood monocytes progressively lose viability when cultured in absence of serum or other stimuli. Their death results from internally regulated process (apoptosis). Addition to the culture of activating factors, such as TNF-alpha dramatically improves cell survival and prevents DNA fragmentation. Propidium iodide (PI) staining is used to measure apoptosis as follows. Monocytes are cultured for 48 hours in polypropylene tubes in serum-free medium (positive control), in the presence of 100 ng/ml TNF-alpha (negative control), and in the presence of varying concentrations of the compound to be tested. Cells are suspended at a concentration of 2×10^6 /ml in PBS containing PI at a

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final concentration of 5 µg/ml, and then incubated at room temperature for 5 minutes before FACScan analysis. PI uptake has been demonstrated to correlate with DNA fragmentation in this experimental paradigm.

- 5 Effect on cytokine release. An important function of monocytes/macrophages is their regulatory activity on other cellular populations of the immune system through the release of cytokines after stimulation. An ELISA to measure cytokine release is performed as follows. Human monocytes are incubated at a density of 5×10^5 cells/ml with increasing concentrations of agonists or antagonists of the invention and under the same conditions, but
10 in the absence of agonists or antagonists. For IL-12 production, the cells are primed overnight with IFN (100 U/ml) in presence of agonist or antagonist of the invention. LPS (10 ng/ml) is then added. Conditioned media are collected after 24h and kept frozen until use. Measurement of TNF-alpha, IL-10, MCP-1 and IL-8 is then performed using a commercially available ELISA kit (e. g, R & D Systems (Minneapolis, MN)) and applying the standard
15 protocols provided with the kit.

- Oxidative burst. Purified monocytes are plated in 96-w plate at 2×10^5 cell/well. Increasing concentrations of agonists or antagonists of the invention are added to the wells in a total volume of 0.2 ml culture medium (RPMI 1640 + 10% FCS, glutamine and antibiotics). After
20 3 days incubation, the plates are centrifuged and the medium is removed from the wells. To the macrophage monolayers, 0.2 ml per well of phenol red solution (140 mM NaCl, 10 mM potassium phosphate buffer pH 7.0, 5.5 mM dextrose, 0.56 mM phenol red and 19 U/ml of HRPO) is added, together with the stimulant (200 nM PMA). The plates are incubated at 37°C for 2 hours and the reaction is stopped by adding 20 µl 1N NaOH per well. The
25 absorbance is read at 610 nm. To calculate the amount of H_2O_2 produced by the macrophages, a standard curve of a H_2O_2 solution of known molarity is performed for each experiment.

- The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test
30 the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

Example 24: Biological Effects of Agonists or Antagonists of the Invention

Astrocyte and Neuronal Assays.

5 Agonists or antagonists of the invention, expressed in *Escherichia coli* and purified as described above, can be tested for activity in promoting the survival, neurite outgrowth, or phenotypic differentiation of cortical neuronal cells and for inducing the proliferation of glial fibrillary acidic protein immunopositive cells, astrocytes. The selection of cortical cells for the bioassay is based on the prevalent expression of FGF-1 and FGF-2 in cortical structures
10 and on the previously reported enhancement of cortical neuronal survival resulting from FGF-2 treatment. A thymidine incorporation assay, for example, can be used to elucidate an agonist or antagonist of the invention's activity on these cells.

Moreover, previous reports describing the biological effects of FGF-2 (basic FGF) on cortical or hippocampal neurons *in vitro* have demonstrated increases in both neuron survival
15 and neurite outgrowth (Walicke et al., "Fibroblast growth factor promotes survival of dissociated hippocampal neurons and enhances neurite extension." *Proc. Natl. Acad. Sci. USA* 83:3012-3016. (1986), assay herein incorporated by reference in its entirety). However, reports from experiments done on PC-12 cells suggest that these two responses are not necessarily synonymous and may depend on not only which FGF is being tested but also on
20 which receptor(s) are expressed on the target cells. Using the primary cortical neuronal culture paradigm, the ability of an agonist or antagonist of the invention to induce neurite outgrowth can be compared to the response achieved with FGF-2 using, for example, a thymidine incorporation assay.

25 **Fibroblast and endothelial cell assays.**

Human lung fibroblasts are obtained from Clonetics (San Diego, CA) and maintained in growth media from Clonetics. Dermal microvascular endothelial cells are obtained from Cell Applications (San Diego, CA). For proliferation assays, the human lung fibroblasts and dermal microvascular endothelial cells can be cultured at 5,000 cells/well in a 96-well plate
30 for one day in growth medium. The cells are then incubated for one day in 0.1% BSA basal medium. After replacing the medium with fresh 0.1% BSA medium, the cells are incubated with the test proteins for 3 days. Alamar Blue (Alamar Biosciences, Sacramento, CA) is

added to each well to a final concentration of 10%. The cells are incubated for 4 hr. Cell viability is measured by reading in a CytoFluor fluorescence reader. For the PGE₂ assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or agonists or antagonists of the invention with or without IL-1 α for 24 hours. The supernatants are collected and assayed for PGE₂ by EIA kit (Cayman, Ann Arbor, MI). For the IL-6 assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or with or without agonists or antagonists of the invention IL-1 α for 24 hours. The supernatants are collected and assayed for IL-6 by ELISA kit (Endogen, Cambridge, MA).

Human lung fibroblasts are cultured with FGF-2 or agonists or antagonists of the invention for 3 days in basal medium before the addition of Alamar Blue to assess effects on growth of the fibroblasts. FGF-2 should show a stimulation at 10 - 2500 ng/ml which can be used to compare stimulation with agonists or antagonists of the invention.

Parkinson Models.

The loss of motor function in Parkinson's disease is attributed to a deficiency of striatal dopamine resulting from the degeneration of the nigrostriatal dopaminergic projection neurons. An animal model for Parkinson's that has been extensively characterized involves the systemic administration of 1-methyl-4 phenyl 1,2,3,6-tetrahydropyridine (MPTP). In the CNS, MPTP is taken-up by astrocytes and catabolized by monoamine oxidase B to 1-methyl-4-phenyl pyridine (MPP⁺) and released. Subsequently, MPP⁺ is actively accumulated in dopaminergic neurons by the high-affinity reuptake transporter for dopamine. MPP⁺ is then concentrated in mitochondria by the electrochemical gradient and selectively inhibits nicotinamide adenine disphosphate: ubiquinone oxidoreductionase (complex I), thereby interfering with electron transport and eventually generating oxygen radicals.

It has been demonstrated in tissue culture paradigms that FGF-2 (basic FGF) has trophic activity towards nigral dopaminergic neurons (Ferrari et al., Dev. Biol. 1989). Recently, Dr. Unsicker's group has demonstrated that administering FGF-2 in gel foam implants in the striatum results in the near complete protection of nigral dopaminergic neurons from the toxicity associated with MPTP exposure (Otto and Unsicker, J. Neuroscience, 1990).

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Based on the data with FGF-2, agonists or antagonists of the invention can be evaluated to determine whether it has an action similar to that of FGF-2 in enhancing dopaminergic neuronal survival *in vitro* and it can also be tested *in vivo* for protection of dopaminergic neurons in the striatum from the damage associated with MPTP treatment. The potential effect of an agonist or antagonist of the invention is first examined *in vitro* in a dopaminergic neuronal cell culture paradigm. The cultures are prepared by dissecting the midbrain floor plate from gestation day 14 Wistar rat embryos. The tissue is dissociated with trypsin and seeded at a density of 200,000 cells/cm² on polyorthinine-laminin coated glass coverslips. The cells are maintained in Dulbecco's Modified Eagle's medium and F12 medium containing hormonal supplements (N1). The cultures are fixed with paraformaldehyde after 8 days *in vitro* and are processed for tyrosine hydroxylase, a specific marker for dopaminergic neurons, immunohistochemical staining. Dissociated cell cultures are prepared from embryonic rats. The culture medium is changed every third day and the factors are also added at that time.

Since the dopaminergic neurons are isolated from animals at gestation day 14, a developmental time which is past the stage when the dopaminergic precursor cells are proliferating, an increase in the number of tyrosine hydroxylase immunopositive neurons would represent an increase in the number of dopaminergic neurons surviving *in vitro*. Therefore, if an agonist or antagonist of the invention acts to prolong the survival of dopaminergic neurons, it would suggest that the agonist or antagonist may be involved in Parkinson's Disease.

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

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Example 25: The Effect of Agonists or Antagonists of the Invention on the Growth of Vascular Endothelial Cells

On day 1, human umbilical vein endothelial cells (HUVEC) are seeded at 2.5×10^4 cells/35 mm dish density in M199 medium containing 4% fetal bovine serum (FBS), 16 units/ml heparin, and 50 units/ml endothelial cell growth supplements (ECGS, Biotechnology,

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Inc.). On day 2, the medium is replaced with M199 containing 10% FBS, 8 units/ml heparin. An agonist or antagonist of the invention, and positive controls, such as VEGF and basic FGF (bFGF) are added, at varying concentrations. On days 4 and 6, the medium is replaced. On day 8, cell number is determined with a Coulter Counter.

- 5 An increase in the number of HUVEC cells indicates that the compound of the invention may proliferate vascular endothelial cells, while a decrease in the number of HUVEC cell indicates that the compound of the invention inhibits vascular endothelial cells.

 The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity
10 of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 26: Rat Corneal Wound Healing Model

 This animal model shows the effect of an agonist or antagonist of the invention on
15 neovascularization. The experimental protocol includes:

- a) Making a 1-1.5 mm long incision from the center of cornea into the stromal layer.
- b) Inserting a spatula below the lip of the incision facing the outer corner of the eye.
- 20 c) Making a pocket (its base is 1-1.5 mm from the edge of the eye).
- d) Positioning a pellet, containing 50ng- 5ug of an agonist or antagonist of the invention, within the pocket.
- e) Treatment with an agonist or antagonist of the invention can also be applied
25 topically to the corneal wounds in a dosage range of 20mg - 500mg (daily treatment for five days).

 The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

Example 27: Diabetic Mouse and Glucocorticoid-Impaired Wound Healing Models

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A. Diabetic db+/db+ Mouse Model.

To demonstrate that an agonist or antagonist of the invention accelerates the healing process, the genetically diabetic mouse model of wound healing is used. The full thickness wound healing model in the db+/db+ mouse is a well characterized, clinically relevant and reproducible model of impaired wound healing. Healing of the diabetic wound is dependent on formation of granulation tissue and re-epithelialization rather than contraction (Gartner, M.H. *et al.*, *J. Surg. Res.* 52:389 (1992); Greenhalgh, D.G. *et al.*, *Am. J. Pathol.* 136:1235 (1990)).

The diabetic animals have many of the characteristic features observed in Type II diabetes mellitus. Homozygous (db+/db+) mice are obese in comparison to their normal heterozygous (db+/+m) littermates. Mutant diabetic (db+/db+) mice have a single autosomal recessive mutation on chromosome 4 (db+) (Coleman *et al.* *Proc. Natl. Acad. Sci. USA* 77:283-293 (1982)). Animals show polyphagia, polydipsia and polyuria. Mutant diabetic mice (db+/db+) have elevated blood glucose, increased or normal insulin levels, and suppressed cell-mediated immunity (Mandel *et al.*, *J. Immunol.* 120:1375 (1978); Debray-Sachs, M. *et al.*, *Clin. Exp. Immunol.* 51(1):1-7 (1983); Leiter *et al.*, *Am. J. of Pathol.* 114:46-55 (1985)). Peripheral neuropathy, myocardial complications, and microvascular lesions, basement membrane thickening and glomerular filtration abnormalities have been described in these animals (Norido, F. *et al.*, *Exp. Neurol.* 83(2):221-232 (1984); Robertson *et al.*, *Diabetes* 29(1):60-67 (1980); Giacomelli *et al.*, *Lab Invest.* 40(4):460-473 (1979); Coleman, D.L., *Diabetes* 31 (Suppl):1-6 (1982)). These homozygous diabetic mice develop hyperglycemia that is resistant to insulin analogous to human type II diabetes (Mandel *et al.*, *J. Immunol.* 120:1375-1377 (1978)).

The characteristics observed in these animals suggests that healing in this model may be similar to the healing observed in human diabetes (Greenhalgh, *et al.*, *Am. J. of Pathol.* 136:1235-1246 (1990)).

Genetically diabetic female C57BL/KsJ (db+/db+) mice and their non-diabetic (db+/+m) heterozygous littermates are used in this study (Jackson Laboratories). The animals are purchased at 6 weeks of age and are 8 weeks old at the beginning of the study. Animals are individually housed and received food and water ad libitum. All manipulations are performed using aseptic techniques. The experiments are conducted according to the

rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

Wounding protocol is performed according to previously reported methods (Tsuboi, R. and Rifkin, D.B., *J. Exp. Med.* 172:245-251 (1990)). Briefly, on the day of wounding, animals are anesthetized with an intraperitoneal injection of Avertin (0.01 mg/mL), 2,2,2-tribromoethanol and 2-methyl-2-butanol dissolved in deionized water. The dorsal region of the animal is shaved and the skin washed with 70% ethanol solution and iodine. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is then created using a Keyes tissue punch. Immediately following wounding, the surrounding skin is gently stretched to eliminate wound expansion. The wounds are left open for the duration of the experiment. Application of the treatment is given topically for 5 consecutive days commencing on the day of wounding. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

Wounds are visually examined and photographed at a fixed distance at the day of surgery and at two day intervals thereafter. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

An agonist or antagonist of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology and immunohistochemistry. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

Three groups of 10 animals each (5 diabetic and 5 non-diabetic controls) are evaluated: 1) Vehicle placebo control, 2) untreated group, and 3) treated group.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total square area of the wound. Contraction is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is 64mm², the corresponding size of the dermal punch. Calculations are made using the following formula:

[Open area on day 8] - [Open area on day 1] / [Open area on day 1]

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned
5 perpendicular to the wound surface (5mm) and cut using a Reichert-Jung microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds are used to assess whether the healing process and the morphologic appearance of the repaired skin is altered by treatment with an agonist or antagonist of the invention. This assessment included verification of the presence
10 of cell accumulation, inflammatory cells, capillaries, fibroblasts, re-epithelialization and epidermal maturity (Greenhalgh, D.G. *et al.*, *Am. J. Pathol.* 136:1235 (1990)). A calibrated lens micrometer is used by a blinded observer.

Tissue sections are also stained immunohistochemically with a polyclonal rabbit anti-human keratin antibody using ABC Elite detection system. Human skin is used as a positive tissue
15 control while non-immune IgG is used as a negative control. Keratinocyte growth is determined by evaluating the extent of reepithelialization of the wound using a calibrated lens micrometer.

Proliferating cell nuclear antigen/cyclin (PCNA) in skin specimens is demonstrated by using anti-PCNA antibody (1:50) with an ABC Elite detection system. Human colon
20 cancer served as a positive tissue control and human brain tissue is used as a negative tissue control. Each specimen included a section with omission of the primary antibody and substitution with non-immune mouse IgG. Ranking of these sections is based on the extent of proliferation on a scale of 0-8, the lower side of the scale reflecting slight proliferation to the higher side reflecting intense proliferation.

25 Experimental data are analyzed using an unpaired t test. A p value of < 0.05 is considered significant.

B. Steroid Impaired Rat Model

The inhibition of wound healing by steroids has been well documented in various *in*
30 *vitro* and *in vivo* systems (Wahl, Glucocorticoids and Wound healing. In: Anti-Inflammatory Steroid Action: Basic and Clinical Aspects. 280-302 (1989); Wahlet *al.*, *J. Immunol.* 115: 476-481 (1975); Werb *et al.*, *J. Exp. Med.* 147:1684-1694 (1978)). Glucocorticoids retard

wound healing by inhibiting angiogenesis, decreasing vascular permeability (Ebert *et al.*, *An. Intern. Med.* 37:701-705 (1952)), fibroblast proliferation, and collagen synthesis (Beck *et al.*, *Growth Factors*. 5: 295-304 (1991); Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978)) and producing a transient reduction of circulating monocytes (Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978); Wahl, "Glucocorticoids and wound healing", *In: Antiinflammatory Steroid Action: Basic and Clinical Aspects*, Academic Press, New York, pp. 280-302 (1989)). The systemic administration of steroids to impaired wound healing is a well establish phenomenon in rats (Beck *et al.*, *Growth Factors*. 5: 295-304 (1991); Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978); Wahl, "Glucocorticoids and wound healing", *In: Antiinflammatory Steroid Action: Basic and Clinical Aspects*, Academic Press, New York, pp. 280-302 (1989); Pierce *et al.*, *Proc. Natl. Acad. Sci. USA* 86: 2229-2233 (1989)).

To demonstrate that an agonist or antagonist of the invention can accelerate the healing process, the effects of multiple topical applications of the agonist or antagonist on full thickness excisional skin wounds in rats in which healing has been impaired by the systemic administration of methylprednisolone is assessed.

Young adult male Sprague Dawley rats weighing 250-300 g (Charles River Laboratories) are used in this example. The animals are purchased at 8 weeks of age and are 9 weeks old at the beginning of the study. The healing response of rats is impaired by the systemic administration of methylprednisolone (17mg/kg/rat intramuscularly) at the time of wounding. Animals are individually housed and received food and water *ad libitum*. All manipulations are performed using aseptic techniques. This study is conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

The wounding protocol is followed according to section A, above. On the day of wounding, animals are anesthetized with an intramuscular injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). The dorsal region of the animal is shaved and the skin washed with 70% ethanol and iodine solutions. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is created using a Keyes tissue punch. The wounds are left open for the duration of the experiment. Applications of the testing materials are given topically once a day for 7 consecutive days commencing on the day of wounding and subsequent to methylprednisolone administration. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

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Wounds are visually examined and photographed at a fixed distance at the day of wounding and at the end of treatment. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

The agonist or antagonist of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

Four groups of 10 animals each (5 with methylprednisolone and 5 without glucocorticoid) are evaluated: 1) Untreated group 2) Vehicle placebo control 3) treated groups.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total area of the wound. Closure is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is 64mm², the corresponding size of the dermal punch. Calculations are made using the following formula:

$$[\text{Open area on day 8}] - [\text{Open area on day 1}] / [\text{Open area on day 1}]$$

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using an Olympus microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds allows assessment of whether the healing process and the morphologic appearance of the repaired skin is improved by treatment with an agonist or antagonist of the invention. A calibrated lens micrometer is used by a blinded observer to determine the distance of the wound gap.

Experimental data are analyzed using an unpaired t test. A p value of < 0.05 is considered significant.

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The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

5

Example 28: Lymphadema Animal Model

The purpose of this experimental approach is to create an appropriate and consistent lymphedema model for testing the therapeutic effects of an agonist or antagonist of the invention in lymphangiogenesis and re-establishment of the lymphatic circulatory system in the rat hind limb. Effectiveness is measured by swelling volume of the affected limb, quantification of the amount of lymphatic vasculature, total blood plasma protein, and histopathology. Acute lymphedema is observed for 7-10 days. Perhaps more importantly, the chronic progress of the edema is followed for up to 3-4 weeks.

15 Prior to beginning surgery, blood sample is drawn for protein concentration analysis. Male rats weighing approximately ~350g are dosed with Pentobarbital. Subsequently, the right legs are shaved from knee to hip. The shaved area is swabbed with gauze soaked in 70% EtOH. Blood is drawn for serum total protein testing. Circumference and volumetric measurements are made prior to injecting dye into paws after marking 2 measurement levels 20 (0.5 cm above heel, at mid-pt of dorsal paw). The intradermal dorsum of both right and left paws are injected with 0.05 ml of 1% Evan's Blue. Circumference and volumetric measurements are then made following injection of dye into paws.

Using the knee joint as a landmark, a mid-leg inguinal incision is made circumferentially allowing the femoral vessels to be located. Forceps and hemostats are used 25 to dissect and separate the skin flaps. After locating the femoral vessels, the lymphatic vessel that runs along side and underneath the vessel(s) is located. The main lymphatic vessels in this area are then electrically coagulated or suture ligated.

Using a microscope, muscles in back of the leg (near the semitendinosus and adductors) are bluntly dissected. The popliteal lymph node is then located. The 2 proximal 30 and 2 distal lymphatic vessels and distal blood supply of the popliteal node are then and ligated by suturing. The popliteal lymph node, and any accompanying adipose tissue, is then removed by cutting connective tissues.

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Care is taken to control any mild bleeding resulting from this procedure. After lymphatics are occluded, the skin flaps are sealed by using liquid skin (Vetbond) (AJ Buck). The separated skin edges are sealed to the underlying muscle tissue while leaving a gap of ~0.5 cm around the leg. Skin also may be anchored by suturing to underlying muscle when
5 necessary.

To avoid infection, animals are housed individually with mesh (no bedding). Recovering animals are checked daily through the optimal edematous peak, which typically occurred by day 5-7. The plateau edematous peak are then observed. To evaluate the intensity of the lymphedema, the circumference and volumes of 2 designated places on each
10 paw before operation and daily for 7 days are measured. The effect plasma proteins on lymphedema is determined and whether protein analysis is a useful testing perimeter is also investigated. The weights of both control and edematous limbs are evaluated at 2 places. Analysis is performed in a blind manner.

Circumference Measurements: Under brief gas anesthetic to prevent limb movement,
15 a cloth tape is used to measure limb circumference. Measurements are done at the ankle bone and dorsal paw by 2 different people then those 2 readings are averaged. Readings are taken from both control and edematous limbs.

Volumetric Measurements: On the day of surgery, animals are anesthetized with Pentobarbital and are tested prior to surgery. For daily volumetrics animals are under brief
20 halothane anesthetic (rapid immobilization and quick recovery), both legs are shaved and equally marked using waterproof marker on legs. Legs are first dipped in water, then dipped into instrument to each marked level then measured by Buxco edema software(Chen/Victor). Data is recorded by one person, while the other is dipping the limb to marked area.

Blood-plasma protein measurements: Blood is drawn, spun, and serum separated
25 prior to surgery and then at conclusion for total protein and Ca²⁺ comparison.

Limb Weight Comparison: After drawing blood, the animal is prepared for tissue collection. The limbs are amputated using a quillitine, then both experimental and control legs are cut at the ligature and weighed. A second weighing is done as the tibio-cacaneal joint is disarticulated and the foot is weighed.

30 Histological Preparations: The transverse muscle located behind the knee (popliteal) area is dissected and arranged in a metal mold, filled with freezeGel, dipped into cold

methylbutane, placed into labeled sample bags at - 80EC until sectioning. Upon sectioning, the muscle is observed under fluorescent microscopy for lymphatics..

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

Example 29: Suppression of TNF alpha-induced adhesion molecule expression by a Agonist or Antagonist of the Invention

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Tumor necrosis factor alpha (TNF-a), a potent proinflammatory cytokine, is a stimulator of all three CAMs on endothelial cells and may be involved in a wide variety of inflammatory responses, often resulting in a pathological outcome.

The potential of an agonist or antagonist of the invention to mediate a suppression of TNF-a induced CAM expression can be examined. A modified ELISA assay which uses ECs as a solid phase absorbent is employed to measure the amount of CAM expression on TNF-a treated ECs when co-stimulated with a member of the FGF family of proteins.

To perform the experiment, human umbilical vein endothelial cell (HUVEC) cultures are obtained from pooled cord harvests and maintained in growth medium (EGM-2; Clonetics, San Diego, CA) supplemented with 10% FCS and 1% penicillin/streptomycin in a 37 degree C humidified incubator containing 5% CO₂. HUVECs are seeded in 96-well plates at concentrations of 1 x 10⁴ cells/well in EGM medium at 37 degree C for 18-24 hrs or until confluent. The monolayers are subsequently washed 3 times with a serum-free solution

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of RPMI-1640 supplemented with 100 U/ml penicillin and 100 mg/ml streptomycin, and treated with a given cytokine and/or growth factor(s) for 24 h at 37 degree C. Following incubation, the cells are then evaluated for CAM expression.

Human Umbilical Vein Endothelial cells (HUVECs) are grown in a standard 96 well plate to confluence. Growth medium is removed from the cells and replaced with 90 ul of 199 Medium (10% FBS). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10 ul volumes). Plates are incubated at 37 degree C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100 µl of 0.1% paraformaldehyde-PBS(with Ca++ and Mg++) is added to each well. Plates are held at 4°C for 30 min.

Fixative is then removed from the wells and wells are washed 1X with PBS(+Ca,Mg)+0.5% BSA and drained. Do not allow the wells to dry. Add 10 µl of diluted primary antibody to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10 µg/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA.

Then add 20 µl of diluted ExtrAvidin-Alkaline Phosphatase (1:5,000 dilution) to each well and incubated at 37°C for 30 min. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA. 1 tablet of p-Nitrophenol Phosphate pNPP is dissolved in 5 ml of glycine buffer (pH 10.4). 100 µl of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphatase in glycine buffer: $1:5,000 (10^0) > 10^{-0.5} > 10^{-1} > 10^{-1.5}$. 5 µl of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100 µl of pNPP reagent must then be added to each of the standard wells. The plate must be incubated at 37°C for 4h. A volume of 50 µl of 3M NaOH is added to all wells. The results are quantified on a plate reader at 405 nm. The background subtraction option is used on blank wells filled with glycine buffer only. The template is set up to indicate the concentration of AP-conjugate in each standard well [5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

Example 30: TAQMAN

Quantitative PCR (QPCR). Total RNA from cells in culture are extracted by Trizol
5 separation as recommended by the supplier (LifeTechnologies). (Total RNA is treated with
DNase I (Life Technologies) to remove any contaminating genomic DNA before reverse
transcription.) Total RNA (50 ng) is used in a one-step, 50ul, RT-QPCR, consisting of
Taqman Buffer A (Perkin-Elmer; 50 mM KCl/10 mM Tris, pH 8.3), 5.5 mM MgCl₂, 240 μM
each dNTP, 0.4 units RNase inhibitor(Promega), 8%glycerol, 0.012% Tween-20, 0.05%
10 gelatin, 0.3uM primers, 0.1uM probe, 0.025units Amplitaq Gold (Perkin-Elmer) and 2.5 units
Superscript II reverse transcriptase (Life Technologies). As a control for genomic
contamination, parallel reactions are setup without reverse transcriptase. The relative
abundance of (unknown) and 18S RNAs are assessed by using the Applied Biosystems Prism
7700 Sequence Detection System (Livak, K. J., Flood, S. J., Marmaro, J., Giusti, W. &
15 Deetz, K. (1995) PCR Methods Appl. 4, 357-362). Reactions are carried out at 48°C for 30
min, 95°C for 10 min, followed by 40 cycles of 95°C for 15s, 60°C for 1 min. Reactions are
performed in triplicate.

Primers (f & r) and FRET probes sets are designed using Primer Express Software
(Perkin-Elmer). Probes are labeled at the 5'-end with the reporter dye 6-FAM and on the 3'-
20 end with the quencher dye TAMRA (Biosource International, Camarillo, CA or Perkin-
Elmer).

***Example 31: Production Of Polypeptide of the Invention For High-
Throughput Screening Assays***

25

The following protocol produces a supernatant containing polypeptide of the present
invention to be tested. This supernatant can then be used in the Screening Assays described
in Examples 33-42.

First, dilute Poly-D-Lysine (644 587 Boehringer-Mannheim) stock solution (1mg/ml
30 in PBS) 1:20 in PBS (w/o calcium or magnesium 17-516F Biowhittaker) for a working
solution of 50ug/ml. Add 200 ul of this solution to each well (24 well plates) and incubate at
RT for 20 minutes. Be sure to distribute the solution over each well (note: a 12-channel

pipetter may be used with tips on every other channel). Aspirate off the Poly-D-Lysine solution and rinse with 1ml PBS (Phosphate Buffered Saline). The PBS should remain in the well until just prior to plating the cells and plates may be poly-lysine coated in advance for up to two weeks.

- 5 Plate 293T cells (do not carry cells past P+20) at 2×10^5 cells/well in .5ml DMEM(Dulbecco's Modified Eagle Medium)(with 4.5 G/L glucose and L-glutamine (12-604F Biowhittaker))/10% heat inactivated FBS(14-503F Biowhittaker)/1x Penstrep(17-602E Biowhittaker). Let the cells grow overnight.

- The next day, mix together in a sterile solution basin: 300 ul Lipofectamine (18324-012 Gibco/BRL) and 5ml Optimem I (31985070 Gibco/BRL)/96-well plate. With a small volume multi-channel pipetter, aliquot approximately 2ug of an expression vector containing a polynucleotide insert, produced by the methods described in Examples 8-10, into an appropriately labeled 96-well round bottom plate. With a multi-channel pipetter, add 50ul of the Lipofectamine/Optimem I mixture to each well. Pipette up and down gently to mix.
- 15 Incubate at RT 15-45 minutes. After about 20 minutes, use a multi-channel pipetter to add 150ul Optimem I to each well. As a control, one plate of vector DNA lacking an insert should be transfected with each set of transfections.

- Preferably, the transfection should be performed by tag-teaming the following tasks. By tag-teaming, hands on time is cut in half, and the cells do not spend too much time on PBS. First, person A aspirates off the media from four 24-well plates of cells, and then person B rinses each well with .5-1ml PBS. Person A then aspirates off PBS rinse, and person B, using a 12-channel pipetter with tips on every other channel, adds the 200ul of DNA/Lipofectamine/Optimem I complex to the odd wells first, then to the even wells, to each row on the 24-well plates. Incubate at 37 degree C for 6 hours.
- 20

- 25 While cells are incubating, prepare appropriate media, either 1%BSA in DMEM with 1x penstrep, or HGS CHO-5 media (116.6 mg/L of CaCl_2 (anhyd); 0.00130 mg/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$; 0.050 mg/L of $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$; 0.417 mg/L of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$; 311.80 mg/L of Kcl; 28.64 mg/L of MgCl_2 ; 48.84 mg/L of MgSO_4 ; 6995.50 mg/L of NaCl; 2400.0 mg/L of NaHCO_3 ; 62.50 mg/L of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$; 71.02 mg/L of Na_2HPO_4 ; .4320 mg/L of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$; .002 mg/L of Arachidonic Acid ; 1.022 mg/L of Cholesterol; .070 mg/L of DL-alpha-Tocopherol-Acetate; 0.0520 mg/L of Linoleic Acid; 0.010 mg/L of Linolenic Acid; 0.010 mg/L of Myristic Acid; 0.010 mg/L of Oleic Acid; 0.010 mg/L of Palmitric Acid; 0.010 mg/L
- 30

of Palmitic Acid; 100 mg/L of Pluronic F-68; 0.010 mg/L of Stearic Acid; 2.20 mg/L of Tween 80; 4551 mg/L of D-Glucose; 130.85 mg/ml of L- Alanine; 147.50 mg/ml of L- Arginine-HCL; 7.50 mg/ml of L-Asparagine-H₂O; 6.65 mg/ml of L-Aspartic Acid; 29.56 mg/ml of L-Cystine-2HCL-H₂O; 31.29 mg/ml of L-Cystine-2HCL; 7.35 mg/ml of L-
5 Glutamic Acid; 365.0 mg/ml of L-Glutamine; 18.75 mg/ml of Glycine; 52.48 mg/ml of L- Histidine-HCL-H₂O; 106.97 mg/ml of L-Isoleucine; 111.45 mg/ml of L-Leucine; 163.75 mg/ml of L-Lysine HCL; 32.34 mg/ml of L-Methionine; 68.48 mg/ml of L-Phenylalanine; 40.0 mg/ml of L-Proline; 26.25 mg/ml of L-Serine; 101.05 mg/ml of L-Threonine; 19.22 mg/ml of L-Tryptophan; 91.79 mg/ml of L-Tyrosine-2Na-2H₂O; and 99.65 mg/ml of L-
10 Valine; 0.0035 mg/L of Biotin; 3.24 mg/L of D-Ca Pantothenate; 11.78 mg/L of Choline Chloride; 4.65 mg/L of Folic Acid; 15.60 mg/L of i-Inositol; 3.02 mg/L of Niacinamide; 3.00 mg/L of Pyridoxal HCL; 0.031 mg/L of Pyridoxine HCL; 0.319 mg/L of Riboflavin; 3.17 mg/L of Thiamine HCL; 0.365 mg/L of Thymidine; 0.680 mg/L of Vitamin B12; 25 mM of HEPES Buffer; 2.39 mg/L of Na Hypoxanthine; 0.105 mg/L of Lipoic Acid; 0.081 mg/L of
15 Sodium Putrescine-2HCL; 55.0 mg/L of Sodium Pyruvate; 0.0067 mg/L of Sodium Selenite; 20uM of Ethanolamine; 0.122 mg/L of Ferric Citrate; 41.70 mg/L of Methyl-B-Cyclodextrin complexed with Linoleic Acid; 33.33 mg/L of Methyl-B-Cyclodextrin complexed with Oleic Acid; 10 mg/L of Methyl-B-Cyclodextrin complexed with Retinal Acetate. Adjust osmolarity to 327 mOsm) with 2mm glutamine and 1x penstrep. (BSA (81-068-3 Bayer)
20 100gm dissolved in 1L DMEM for a 10% BSA stock solution). Filter the media and collect 50 ul for endotoxin assay in 15ml polystyrene conical.

The transfection reaction is terminated, preferably by tag-teaming, at the end of the incubation period. Person A aspirates off the transfection media, while person B adds 1.5ml appropriate media to each well. Incubate at 37 degree C for 45 or 72 hours depending on the
25 media used: 1%BSA for 45 hours or CHO-5 for 72 hours.

On day four, using a 300ul multichannel pipetter, aliquot 600ul in one 1ml deep well plate and the remaining supernatant into a 2ml deep well. The supernatants from each well can then be used in the assays described in Examples 33-40.

It is specifically understood that when activity is obtained in any of the assays
30 described below using a supernatant, the activity originates from either the polypeptide of the present invention directly (e.g., as a secreted protein) or by polypeptide of the present invention inducing expression of other proteins, which are then secreted into the supernatant.

Thus, the invention further provides a method of identifying the protein in the supernatant characterized by an activity in a particular assay.

Example 32: Construction of GAS Reporter Construct

5

One signal transduction pathway involved in the differentiation and proliferation of cells is called the Jaks-STATs pathway. Activated proteins in the Jaks-STATs pathway bind to gamma activation site "GAS" elements or interferon-sensitive responsive element ("ISRE"), located in the promoter of many genes. The binding of a protein to these elements alter the expression of the associated gene.

10

GAS and ISRE elements are recognized by a class of transcription factors called Signal Transducers and Activators of Transcription, or "STATs." There are six members of the STATs family. Stat1 and Stat3 are present in many cell types, as is Stat2 (as response to IFN-alpha is widespread). Stat4 is more restricted and is not in many cell types though it has been found in T helper class I, cells after treatment with IL-12. Stat5 was originally called mammary growth factor, but has been found at higher concentrations in other cells including myeloid cells. It can be activated in tissue culture cells by many cytokines.

15

The STATs are activated to translocate from the cytoplasm to the nucleus upon tyrosine phosphorylation by a set of kinases known as the Janus Kinase ("Jaks") family. Jaks represent a distinct family of soluble tyrosine kinases and include Tyk2, Jak1, Jak2, and Jak3. These kinases display significant sequence similarity and are generally catalytically inactive in resting cells.

20

The Jaks are activated by a wide range of receptors summarized in the Table below. (Adapted from review by Schidler and Darnell, Ann. Rev. Biochem. 64:621-51 (1995).) A cytokine receptor family, capable of activating Jaks, is divided into two groups: (a) Class 1 includes receptors for IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-11, IL-12, IL-15, Epo, PRL, GH, G-CSF, GM-CSF, LIF, CNTF, and thrombopoietin; and (b) Class 2 includes IFN-a, IFN-g, and IL-10. The Class 1 receptors share a conserved cysteine motif (a set of four conserved cysteines and one tryptophan) and a WSXWS motif (a membrane proximal region encoding Trp-Ser-Xxx-Trp-Ser (SEQ ID NO: 8556)).

25

30

Thus, on binding of a ligand to a receptor, Jaks are activated, which in turn activate STATs, which then translocate and bind to GAS elements. This entire process is

encompassed in the Jaks-STATs signal transduction pathway.

Therefore, activation of the Jaks-STATs pathway, reflected by the binding of the GAS or the ISRE element, can be used to indicate proteins involved in the proliferation and differentiation of cells. For example, growth factors and cytokines are known to activate the
5 Jaks-STATs pathway. (See Table below.) Thus, by using GAS elements linked to reporter molecules, activators of the Jaks-STATs pathway can be identified.

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	<u>Ligand</u>	<u>JAKs</u>				<u>STATS GAS(elements) or ISRE</u>	
		<u>tyk2</u>	<u>Jak1</u>	<u>Jak2</u>	<u>Jak3</u>		
	<u>IFN family</u>						
5	IFN-a/B	+	+	-	-	1,2,3	ISRE
	IFN-g (IRF1>Lys6>IFP)		+	+	-	1	GAS
	IL-10	+	?	?	-	1,3	
10	<u>gp130 family</u>						
	IL-6 (Pleiotrohic) (IRF1>Lys6>IFP)	+	+	+	?	1,3	GAS
	IL-11(Pleiotrohic)	?	+	?	?	1,3	
	OnM(Pleiotrohic)	?	+	+	?	1,3	
15	LIF(Pleiotrohic)	?	+	+	?	1,3	
	CNTF(Pleiotrohic)	-/+	+	+	?	1,3	
	G-CSF(Pleiotrohic)	?	+	?	?	1,3	
	IL-12(Pleiotrohic)	+	-	+	+	1,3	
20	<u>g-C family</u>						
	IL-2 (lymphocytes)	-	+	-	+	1,3,5	GAS
	IL-4 (lymph/myeloid) >>Ly6)(IgH)	-	+	-	+	6	GAS (IRF1 = IFP
	IL-7 (lymphocytes)	-	+	-	+	5	GAS
25	IL-9 (lymphocytes)	-	+	-	+	5	GAS
	IL-13 (lymphocyte)	-	+	?	?	6	GAS
	IL-15	?	+	?	+	5	GAS
	<u>gp140 family</u>						
30	IL-3 (myeloid) (IRF1>IFP>>Ly6)	-	-	+	-	5	GAS
	IL-5 (myeloid)	-	-	+	-	5	GAS
	GM-CSF (myeloid)	-	-	+	-	5	GAS

Growth hormone family

	GH	?	-	+	-	5	
	PRL	?	+/-	+	-	1,3,5	
5	EPO	?	-	+	-	5	GAS(B-
	CAS>IRF1=IFP>>Ly6)						

Receptor Tyrosine Kinases

10	EGF	?	+	+	-	1,3	GAS (IRF1)
	PDGF	?	+	+	-	1,3	
	CSF-1	?	+	+	-	1,3	GAS (not IRF1)

To construct a synthetic GAS containing promoter element, which is used in the Biological Assays described in Examples 33-34, a PCR based strategy is employed to generate a GAS-SV40 promoter sequence. The 5' primer contains four tandem copies of the GAS binding site found in the IRF1 promoter and previously demonstrated to bind STATs upon induction with a range of cytokines (Rothman et al., Immunity 1:457-468 (1994).), although other GAS or ISRE elements can be used instead. The 5' primer also contains 18bp of sequence complementary to the SV40 early promoter sequence and is flanked with an XhoI site. The sequence of the 5' primer is:

5':GCGCCTCGAGATTTCCCGAAATCTAGATTTCCCGAAATGATTTCCCGAAAT
10 GATTTCCCGAAATATCTGCCATCTCAATTAG:3' (SEQ ID NO:8557)

The downstream primer is complementary to the SV40 promoter and is flanked with a Hind III site: 5':GCGGCAAGCTTTTTGCAAAGCCTAGGC:3' (SEQ ID NO:8558)

PCR amplification is performed using the SV40 promoter template present in the B-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI/Hind III and subcloned into BLSK2-. (Stratagene.) Sequencing with forward and reverse primers confirms that the insert contains the following sequence:

5':CTCGAGATTTCCCGAAATCTAGATTTCCCGAAATGATTTCCCGAAATGATT
TCCCGAAATATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACT
CCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTG
20 ACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCTCGGCCTCTGAGCTATTCC
AGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTT:3'
(SEQ ID NO:8559)

With this GAS promoter element linked to the SV40 promoter, a GAS:SEAP2 reporter construct is next engineered. Here, the reporter molecule is a secreted alkaline phosphatase, or "SEAP." Clearly, however, any reporter molecule can be instead of SEAP, in this or in any of the other Examples. Well known reporter molecules that can be used instead of SEAP include chloramphenicol acetyltransferase (CAT), luciferase, alkaline phosphatase, B-galactosidase, green fluorescent protein (GFP), or any protein detectable by an antibody.

30 The above sequence confirmed synthetic GAS-SV40 promoter element is subcloned into the pSEAP-Promoter vector obtained from Clontech using HindIII and XhoI, effectively replacing the SV40 promoter with the amplified GAS:SV40 promoter element, to create the

GAS-SEAP vector. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

Thus, in order to generate mammalian stable cell lines expressing the GAS-SEAP reporter, the GAS-SEAP cassette is removed from the GAS-SEAP vector using SalI and NotI, and inserted into a backbone vector containing the neomycin resistance gene, such as pGFP-1 (Clontech), using these restriction sites in the multiple cloning site, to create the GAS-SEAP/Neo vector. Once this vector is transfected into mammalian cells, this vector can then be used as a reporter molecule for GAS binding as described in Examples 33-34.

Other constructs can be made using the above description and replacing GAS with a different promoter sequence. For example, construction of reporter molecules containing NFK-B and EGR promoter sequences are described in Examples 35 and 36. However, many other promoters can be substituted using the protocols described in these Examples. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be substituted, alone or in combination (e.g., GAS/NF-KB/EGR, GAS/NF-KB, IL-2/NFAT, or NF-KB/GAS). Similarly, other cell lines can be used to test reporter construct activity, such as HELA (epithelial), HUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), HUVAC (aortic), or Cardiomyocyte.

Example 33: High-Throughput Screening Assay for T-cell Activity.

20

The following protocol is used to assess T-cell activity by identifying factors, and determining whether supernate containing a polypeptide of the invention proliferates and/or differentiates T-cells. T-cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 32. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The T-cell used in this assay is Jurkat T-cells (ATCC Accession No. TIB-152), although Molt-3 cells (ATCC Accession No. CRL-1552) and Molt-4 cells (ATCC Accession No. CRL-1582) cells can also be used.

Jurkat T-cells are lymphoblastic CD4+ Th1 helper cells. In order to generate stable cell lines, approximately 2 million Jurkat cells are transfected with the GAS-SEAP/neo vector using DMRIE-C (Life Technologies)(transfection procedure described below). The transfected cells are seeded to a density of approximately 20,000 cells per well and transfectants resistant to 1 mg/ml gentamicin selected. Resistant colonies are expanded and then

tested for their response to increasing concentrations of interferon gamma. The dose response of a selected clone is demonstrated.

Specifically, the following protocol will yield sufficient cells for 75 wells containing 200 ul of cells. Thus, it is either scaled up, or performed in multiple to generate sufficient
5 cells for multiple 96 well plates. Jurkat cells are maintained in RPMI + 10% serum with 1%Pen-Strep. Combine 2.5 mls of OPTI-MEM (Life Technologies) with 10 ug of plasmid DNA in a T25 flask. Add 2.5 ml OPTI-MEM containing 50 ul of DMRIE-C and incubate at room temperature for 15-45 mins.

During the incubation period, count cell concentration, spin down the required
10 number of cells (10^7 per transfection), and resuspend in OPTI-MEM to a final concentration of 10^7 cells/ml. Then add 1ml of 1×10^7 cells in OPTI-MEM to T25 flask and incubate at 37 degree C for 6 hrs. After the incubation, add 10 ml of RPMI + 15% serum.

The Jurkat:GAS-SEAP stable reporter lines are maintained in RPMI + 10% serum, 1 mg/ml Gentacin, and 1% Pen-Strep. These cells are treated with supernatants containing
15 polypeptide of the present invention or polypeptide of the present invention induced polypeptides as produced by the protocol described in Example 31.

On the day of treatment with the supernatant, the cells should be washed and resuspended in fresh RPMI + 10% serum to a density of 500,000 cells per ml. The exact number of cells required will depend on the number of supernatants being screened. For one
20 96 well plate, approximately 10 million cells (for 10 plates, 100 million cells) are required.

Transfer the cells to a triangular reservoir boat, in order to dispense the cells into a 96 well dish, using a 12 channel pipette. Using a 12 channel pipette, transfer 200 ul of cells into each well (therefore adding 100, 000 cells per well).

After all the plates have been seeded, 50 ul of the supernatants are transferred directly
25 from the 96 well plate containing the supernatants into each well using a 12 channel pipette. In addition, a dose of exogenous interferon gamma (0.1, 1.0, 10 ng) is added to wells H9, H10, and H11 to serve as additional positive controls for the assay.

The 96 well dishes containing Jurkat cells treated with supernatants are placed in an incubator for 48 hrs (note: this time is variable between 48-72 hrs). 35 ul samples from each
30 well are then transferred to an opaque 96 well plate using a 12 channel pipette. The opaque plates should be covered (using sellophene covers) and stored at -20 degree C until SEAP assays are performed according to Example 37. The plates containing the remaining treated

cells are placed at 4 degree C and serve as a source of material for repeating the assay on a specific well if desired.

As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate Jurkat T cells. Over 30 fold induction is typically observed in the positive control wells.

The above protocol may be used in the generation of both transient, as well as, stable transfected cells, which would be apparent to those of skill in the art.

Example 34: High-Throughput Screening Assay Identifying Myeloid Activity.

10

The following protocol is used to assess myeloid activity of polypeptide of the present invention by determining whether polypeptide of the present invention proliferates and/or differentiates myeloid cells. Myeloid cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 32. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The myeloid cell used in this assay is U937, a pre-monocyte cell line, although TF-1, HL60, or KG1 can be used.

To transiently transfect U937 cells with the GAS/SEAP/Neo construct produced in Example 32, a DEAE-Dextran method (Kharbanda et. al., 1994, Cell Growth & Differentiation, 5:259-265) is used. First, harvest 2×10^7 U937 cells and wash with PBS. The U937 cells are usually grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin.

Next, suspend the cells in 1 ml of 20 mM Tris-HCl (pH 7.4) buffer containing 0.5 mg/ml DEAE-Dextran, 8 ug GAS-SEAP2 plasmid DNA, 140 mM NaCl, 5 mM KCl, 375 uM $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, 1 mM MgCl_2 , and 675 uM CaCl_2 . Incubate at 37 degrees C for 45 min.

Wash the cells with RPMI 1640 medium containing 10% FBS and then resuspend in 10 ml complete medium and incubate at 37 degree C for 36 hr.

The GAS-SEAP/U937 stable cells are obtained by growing the cells in 400 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 400 ug/ml G418 for couple of passages.

These cells are tested by harvesting 1×10^8 cells (this is enough for ten 96-well plates assay) and wash with PBS. Suspend the cells in 200 ml above described growth medium,

with a final density of 5×10^5 cells/ml. Plate 200 μ l cells per well in the 96-well plate (or 1×10^5 cells/well).

Add 50 μ l of the supernatant prepared by the protocol described in Example 31. Incubate at 37 degree C for 48 to 72 hr. As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate U937 cells. Over 30 fold induction is typically observed in the positive control wells. SEAP assay the supernatant according to the protocol described in Example 37.

Example 35: High-Throughput Screening Assay Identifying Neuronal Activity.

When cells undergo differentiation and proliferation, a group of genes are activated through many different signal transduction pathways. One of these genes, EGR1 (early growth response gene 1), is induced in various tissues and cell types upon activation. The promoter of EGR1 is responsible for such induction. Using the EGR1 promoter linked to reporter molecules, activation of cells can be assessed by polypeptide of the present invention.

Particularly, the following protocol is used to assess neuronal activity in PC12 cell lines. PC12 cells (rat pheochromocytoma cells) are known to proliferate and/or differentiate by activation with a number of mitogens, such as TPA (tetradecanoyl phorbol acetate), NGF (nerve growth factor), and EGF (epidermal growth factor). The EGR1 gene expression is activated during this treatment. Thus, by stably transfecting PC12 cells with a construct containing an EGR promoter linked to SEAP reporter, activation of PC12 cells by polypeptide of the present invention can be assessed.

The EGR/SEAP reporter construct can be assembled by the following protocol. The EGR-1 promoter sequence (-633 to +1)(Sakamoto K et al., Oncogene 6:867-871 (1991)) can be PCR amplified from human genomic DNA using the following primers:

5' GCGCTCGAGGGATGACAGCGATAGAACCCCGG -3' (SEQ ID NO: 8560)

5' GCGAAGCTTCGCGACTCCCCGGATCCGCCTC-3' (SEQ ID NO: 8561)

Using the GAS:SEAP/Neo vector produced in Example 32, EGR1 amplified product can then be inserted into this vector. Linearize the GAS:SEAP/Neo vector using restriction enzymes XhoI/HindIII, removing the GAS/SV40 stuffer. Restrict the EGR1 amplified

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product with these same enzymes. Ligate the vector and the EGR1 promoter.

To prepare 96 well-plates for cell culture, two mls of a coating solution (1:30 dilution of collagen type I (Upstate Biotech Inc. Cat#08-115) in 30% ethanol (filter sterilized)) is added per one 10 cm plate or 50 ml per well of the 96-well plate, and allowed to air dry for 2
5 hr.

PC12 cells are routinely grown in RPMI-1640 medium (Bio Whittaker) containing 10% horse serum (JRH BIOSCIENCES, Cat. # 12449-78P), 5% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 ug/ml streptomycin on a precoated 10 cm tissue culture dish. One to four split is done every three to four days. Cells
10 are removed from the plates by scraping and resuspended with pipetting up and down for more than 15 times.

Transfect the EGR/SEAP/Neo construct into PC12 using the Lipofectamine protocol described in Example 31. EGR-SEAP/PC12 stable cells are obtained by growing the cells in 300 ug/ml G418. The G418-free medium is used for routine growth but every one to two
15 months, the cells should be re-grown in 300 ug/ml G418 for couple of passages.

To assay for neuronal activity, a 10 cm plate with cells around 70 to 80% confluent is screened by removing the old medium. Wash the cells once with PBS (Phosphate buffered saline). Then starve the cells in low serum medium (RPMI-1640 containing 1% horse serum and 0.5% FBS with antibiotics) overnight.

20 The next morning, remove the medium and wash the cells with PBS. Scrape off the cells from the plate, suspend the cells well in 2 ml low serum medium. Count the cell number and add more low serum medium to reach final cell density as 5×10^5 cells/ml.

Add 200 ul of the cell suspension to each well of 96-well plate (equivalent to 1×10^5 cells/well). Add 50 ul supernatant produced by Example 31, 37 degree C for 48 to 72 hr. As
25 a positive control, a growth factor known to activate PC12 cells through EGR can be used, such as 50 ng/ul of Neuronal Growth Factor (NGF). Over fifty-fold induction of SEAP is typically seen in the positive control wells. SEAP assay the supernatant according to Example 37.

30 ***Example 36: High-Throughput Screening Assay for T-cell Activity.***

NF-KB (Nuclear Factor KB) is a transcription factor activated by a wide variety of

agents including the inflammatory cytokines IL-1 and TNF, CD30 and CD40, lymphotoxin-alpha and lymphotoxin-beta, by exposure to LPS or thrombin, and by expression of certain viral gene products. As a transcription factor, NF-KB regulates the expression of genes involved in immune cell activation, control of apoptosis (NF-KB appears to shield cells from apoptosis), B and T-cell development, anti-viral and antimicrobial responses, and multiple stress responses.

In non-stimulated conditions, NF-KB is retained in the cytoplasm with I-KB (Inhibitor KB). However, upon stimulation, I-KB is phosphorylated and degraded, causing NF-KB to shuttle to the nucleus, thereby activating transcription of target genes. Target genes activated by NF-KB include IL-2, IL-6, GM-CSF, ICAM-1 and class 1 MHC.

Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the NF-KB promoter element are used to screen the supernatants produced in Example 31. Activators or inhibitors of NF-KB would be useful in treating, preventing, and/or diagnosing diseases. For example, inhibitors of NF-KB could be used to treat those diseases related to the acute or chronic activation of NF-KB, such as rheumatoid arthritis.

To construct a vector containing the NF-KB promoter element, a PCR based strategy is employed. The upstream primer contains four tandem copies of the NF-KB binding site (GGGGACTTCCCC) (SEQ ID NO:8562), 18 bp of sequence complementary to the 5' end of the SV40 early promoter sequence, and is flanked with an XhoI site:

5':GCGGCCTCGAGGGGACTTCCCCGGGGACTTTCGGGGACTTTCGGGGACTTTC
CATCCTGCCATCTCAATTAG:3' (SEQ ID NO:8563)

The downstream primer is complementary to the 3' end of the SV40 promoter and is flanked with a Hind III site:

5':GCGGCAAGCTTTTGGCAAAGCCTAGGC:3' (SEQ ID NO:8558)

PCR amplification is performed using the SV40 promoter template present in the pB-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI and Hind III and subcloned into BLSK2-. (Stratagene) Sequencing with the T7 and T3 primers confirms the insert contains the following sequence:

5':CTCGAGGGGACTTCCCCGGGGACTTTCGGGGACTTTCGGGGACTTTCATCTG
CCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCC
CTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTAT
TTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGG

AGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTT:3' (SEQ ID NO:8564)

Next, replace the SV40 minimal promoter element present in the pSEAP2-promoter plasmid (Clontech) with this NF-KB/SV40 fragment using XhoI and HindIII. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for
5 mammalian expression systems.

In order to generate stable mammalian cell lines, the NF-KB/SV40/SEAP cassette is removed from the above NF-KB/SEAP vector using restriction enzymes SalI and NotI, and inserted into a vector containing neomycin resistance. Particularly, the NF-KB/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the GFP gene, after restricting pGFP-
10 1 with SalI and NotI.

Once NF-KB/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are created and maintained according to the protocol described in Example 33. Similarly, the method for assaying supernatants with these stable Jurkat T-cells is also described in Example 33. As a positive control, exogenous TNF alpha (0.1, 1, 10 ng) is added to wells H9, H10, and H11,
15 with a 5-10 fold activation typically observed.

Example 37: Assay for SEAP Activity.

As a reporter molecule for the assays described in Examples 33-36, SEAP activity is
20 assayed using the Tropix Phospho-light Kit (Cat. BP-400) according to the following general procedure. The Tropix Phospho-light Kit supplies the Dilution, Assay, and Reaction Buffers used below.

Prime a dispenser with the 2.5x Dilution Buffer and dispense 15 ul of 2.5x dilution buffer into Optiplates containing 35 ul of a supernatant. Seal the plates with a plastic sealer
25 and incubate at 65 degree C for 30 min. Separate the Optiplates to avoid uneven heating.

Cool the samples to room temperature for 15 minutes. Empty the dispenser and prime with the Assay Buffer. Add 50 ml Assay Buffer and incubate at room temperature 5 min. Empty the dispenser and prime with the Reaction Buffer (see the table below). Add 50 ul Reaction Buffer and incubate at room temperature for 20 minutes. Since the intensity of the
30 chemiluminescent signal is time dependent, and it takes about 10 minutes to read 5 plates on luminometer, one should treat 5 plates at each time and start the second set 10 minutes later.

Read the relative light unit in the luminometer. Set H12 as blank, and print the

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results. An increase in chemiluminescence

indicates reporter activity.

Reaction Buffer Formulation:

# of plates	Rxn buffer diluent (ml)	CSPD (ml)
10	60	3
11	65	3.25
12	70	3.5
13	75	3.75
14	80	4
15	85	4.25
16	90	4.5
17	95	4.75
18	100	5
19	105	5.25
20	110	5.5
21	115	5.75
22	120	6
23	125	6.25
24	130	6.5
25	135	6.75
26	140	7
27	145	7.25
28	150	7.5
29	155	7.75
30	160	8
31	165	8.25
32	170	8.5
33	175	8.75
34	180	9
35	185	9.25
36	190	9.5
37	195	9.75
38	200	10
39	205	10.25
40	210	10.5
41	215	10.75
42	220	11

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43	225	11.25
44	230	11.5
45	235	11.75
46	240	12
47	245	12.25
48	250	12.5
49	255	12.75
50	260	13

Example 38: High-Throughput Screening Assay Identifying Changes in Small Molecule Concentration and Membrane Permeability.

5

Binding of a ligand to a receptor is known to alter intracellular levels of small molecules, such as calcium, potassium, sodium, and pH, as well as alter membrane potential. These alterations can be measured in an assay to identify supernatants which bind to receptors of a particular cell. Although the following protocol describes an assay for calcium, this protocol can easily be modified to detect changes in potassium, sodium, pH, membrane potential, or any other small molecule which is detectable by a fluorescent probe.

The following assay uses Fluorometric Imaging Plate Reader ("FLIPR") to measure changes in fluorescent molecules (Molecular Probes) that bind small molecules. Clearly, any fluorescent molecule detecting a small molecule can be used instead of the calcium fluorescent molecule, fluo-4 (Molecular Probes, Inc.; catalog no. F-14202), used here.

For adherent cells, seed the cells at 10,000 -20,000 cells/well in a Co-star black 96-well plate with clear bottom. The plate is incubated in a CO₂ incubator for 20 hours. The adherent cells are washed two times in Biotek washer with 200 ul of HBSS (Hank's Balanced Salt Solution) leaving 100 ul of buffer after the final wash.

A stock solution of 1 mg/ml fluo-4 is made in 10% pluronic acid DMSO. To load the cells with fluo-4, 50 ul of 12 ug/ml fluo-4 is added to each well. The plate is incubated at 37 degrees C in a CO₂ incubator for 60 min. The plate is washed four times in the Biotek washer with HBSS leaving 100 ul of buffer.

For non-adherent cells, the cells are spun down from culture media. Cells are re-suspended to 2-5x10⁶ cells/ml with HBSS in a 50-ml conical tube. 4 ul of 1 mg/ml fluo-4

solution in 10% pluronic acid DMSO is added to each ml of cell suspension. The tube is then placed in a 37 degrees C water bath for 30-60 min. The cells are washed twice with HBSS, resuspended to 1×10^6 cells/ml, and dispensed into a microplate, 100 ul/well. The plate is centrifuged at 1000 rpm for 5 min. The plate is then washed once in Denley Cell Wash with 200 ul, followed by an aspiration step to 100 ul final volume.

For a non-cell based assay, each well contains a fluorescent molecule, such as fluo-4 . The supernatant is added to the well, and a change in fluorescence is detected.

To measure the fluorescence of intracellular calcium, the FLIPR is set for the following parameters: (1) System gain is 300-800 mW; (2) Exposure time is 0.4 second; (3) Camera F/stop is F/2; (4) Excitation is 488 nm; (5) Emission is 530 nm; and (6) Sample addition is 50 ul. Increased emission at 530 nm indicates an extracellular signaling event caused by the a molecule, either polypeptide of the present invention or a molecule induced by polypeptide of the present invention, which has resulted in an increase in the intracellular Ca^{++} concentration.

Example 40: High-Throughput Screening Assay Identifying Tyrosine Kinase Activity.

The Protein Tyrosine Kinases (PTK) represent a diverse group of transmembrane and cytoplasmic kinases. Within the Receptor Protein Tyrosine Kinase (RPTK) group are receptors for a range of mitogenic and metabolic growth factors including the PDGF, FGF, EGF, NGF, HGF and Insulin receptor subfamilies. In addition there are a large family of RPTKs for which the corresponding ligand is unknown. Ligands for RPTKs include mainly secreted small proteins, but also membrane-bound and extracellular matrix proteins.

Activation of RPTK by ligands involves ligand-mediated receptor dimerization, resulting in transphosphorylation of the receptor subunits and activation of the cytoplasmic tyrosine kinases. The cytoplasmic tyrosine kinases include receptor associated tyrosine kinases of the src-family (e.g., src, yes, lck, lyn, fyn) and non-receptor linked and cytosolic protein tyrosine kinases, such as the Jak family, members of which mediate signal transduction triggered by the cytokine superfamily of receptors (e.g., the Interleukins, Interferons, GM-CSF, and Leptin).

Because of the wide range of known factors capable of stimulating tyrosine kinase

activity, identifying whether polypeptide of the present invention or a molecule induced by polypeptide of the present invention is capable of activating tyrosine kinase signal transduction pathways is of interest. Therefore, the following protocol is designed to identify such molecules capable of activating the tyrosine kinase signal transduction pathways.

5 Seed target cells (e.g., primary keratinocytes) at a density of approximately 25,000 cells per well in a 96 well Loprodyne Silent Screen Plates purchased from Nalge Nunc (Naperville, IL). The plates are sterilized with two 30 minute rinses with 100% ethanol, rinsed with water and dried overnight. Some plates are coated for 2 hr with 100 ml of cell culture grade type I collagen (50 mg/ml), gelatin (2%) or polylysine (50 mg/ml), all of which
10 can be purchased from Sigma Chemicals (St. Louis, MO) or 10% Matrigel purchased from Becton Dickinson (Bedford, MA), or calf serum, rinsed with PBS and stored at 4 degree C. Cell growth on these plates is assayed by seeding 5,000 cells/well in growth medium and indirect quantitation of cell number through use of alamarBlue as described by the manufacturer Alamar Biosciences, Inc. (Sacramento, CA) after 48 hr. Falcon plate covers
15 #3071 from Becton Dickinson (Bedford, MA) are used to cover the Loprodyne Silent Screen Plates. Falcon Microtest III cell culture plates can also be used in some proliferation experiments.

 To prepare extracts, A431 cells are seeded onto the nylon membranes of Loprodyne plates (20,000/200ml/well) and cultured overnight in complete medium. Cells are quiesced
20 by incubation in serum-free basal medium for 24 hr. After 5-20 minutes treatment with EGF (60ng/ml) or 50 ul of the supernatant produced in Example 31, the medium was removed and 100 ml of extraction buffer ((20 mM HEPES pH 7.5, 0.15 M NaCl, 1% Triton X-100, 0.1% SDS, 2 mM Na₃VO₄, 2 mM Na₄P₂O₇ and a cocktail of protease inhibitors (# 1836170) obtained from Boehringer Mannheim (Indianapolis, IN) is added to each well and the plate
25 is shaken on a rotating shaker for 5 minutes at 40C. The plate is then placed in a vacuum transfer manifold and the extract filtered through the 0.45 mm membrane bottoms of each well using house vacuum. Extracts are collected in a 96-well catch/assay plate in the bottom of the vacuum manifold and immediately placed on ice. To obtain extracts clarified by centrifugation, the content of each well, after detergent solubilization for 5 minutes, is
30 removed and centrifuged for 15 minutes at 4 degree C at 16,000 x g.

 Test the filtered extracts for levels of tyrosine kinase activity. Although many methods of detecting tyrosine kinase activity are known, one method is described here.

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Generally, the tyrosine kinase activity of a supernatant is evaluated by determining its ability to phosphorylate a tyrosine residue on a specific substrate (a biotinylated peptide). Biotinylated peptides that can be used for this purpose include PSK1 (corresponding to amino acids 6-20 of the cell division kinase cdc2-p34) and PSK2 (corresponding to amino acids 1-17 of gastrin). Both peptides are substrates for a range of tyrosine kinases and are available from Boehringer Mannheim.

The tyrosine kinase reaction is set up by adding the following components in order. First, add 10ul of 5uM Biotinylated Peptide, then 10ul ATP/Mg²⁺ (5mM ATP/50mM MgCl₂), then 10ul of 5x Assay Buffer (40mM imidazole hydrochloride, pH7.3, 40 mM beta-glycerophosphate, 1mM EGTA, 100mM MgCl₂, 5 mM MnCl₂, 0.5 mg/ml BSA), then 5ul of Sodium Vanadate(1mM), and then 5ul of water. Mix the components gently and preincubate the reaction mix at 30 degree C for 2 min. Initiate the reaction by adding 10ul of the control enzyme or the filtered supernatant.

The tyrosine kinase assay reaction is then terminated by adding 10 ul of 120mM EDTA and place the reactions on ice.

Tyrosine kinase activity is determined by transferring 50 ul aliquot of reaction mixture to a microtiter plate (MTP) module and incubating at 37 degree C for 20 min. This allows the streptavidin coated 96 well plate to associate with the biotinylated peptide. Wash the MTP module with 300ul/well of PBS four times. Next add 75 ul of anti-phosphotyrosine antibody conjugated to horse radish peroxidase(anti-P-Tyr-POD(0.5u/ml)) to each well and incubate at 37 degree C for one hour. Wash the well as above.

Next add 100ul of peroxidase substrate solution (Boehringer Mannheim) and incubate at room temperature for at least 5 mins (up to 30 min). Measure the absorbance of the sample at 405 nm by using ELISA reader. The level of bound peroxidase activity is quantitated using an ELISA reader and reflects the level of tyrosine kinase activity.

Example 41: High-Throughput Screening Assay Identifying Phosphorylation Activity.

As a potential alternative and/or complement to the assay of protein tyrosine kinase activity described in Example 40, an assay which detects activation (phosphorylation) of major intracellular signal transduction intermediates can also be used. For example, as

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described below one particular assay can detect tyrosine phosphorylation of the Erk-1 and Erk-2 kinases. However, phosphorylation of other molecules, such as Raf, JNK, p38 MAP, Map kinase kinase (MEK), MEK kinase, Src, Muscle specific kinase (MuSK), IRAK, Tec, and Janus, as well as any other phosphoserine, phosphotyrosine, or phosphothreonine molecule, can be detected by substituting these molecules for Erk-1 or Erk-2 in the following assay.

Specifically, assay plates are made by coating the wells of a 96-well ELISA plate with 0.1ml of protein G (1ug/ml) for 2 hr at room temp, (RT). The plates are then rinsed with PBS and blocked with 3% BSA/PBS for 1 hr at RT. The protein G plates are then treated with 2 commercial monoclonal antibodies (100ng/well) against Erk-1 and Erk-2 (1 hr at RT) (Santa Cruz Biotechnology). (To detect other molecules, this step can easily be modified by substituting a monoclonal antibody detecting any of the above described molecules.) After 3-5 rinses with PBS, the plates are stored at 4 degree C until use.

A431 cells are seeded at 20,000/well in a 96-well Loprodyne filterplate and cultured overnight in growth medium. The cells are then starved for 48 hr in basal medium (DMEM) and then treated with EGF (6ng/well) or 50 ul of the supernatants obtained in Example 31 for 5-20 minutes. The cells are then solubilized and extracts filtered directly into the assay plate.

After incubation with the extract for 1 hr at RT, the wells are again rinsed. As a positive control, a commercial preparation of MAP kinase (10ng/well) is used in place of A431 extract. Plates are then treated with a commercial polyclonal (rabbit) antibody (1ug/ml) which specifically recognizes the phosphorylated epitope of the Erk-1 and Erk-2 kinases (1 hr at RT). This antibody is biotinylated by standard procedures. The bound polyclonal antibody is then quantitated by successive incubations with Europium-streptavidin and Europium fluorescence enhancing reagent in the Wallac DELFIA instrument (time-resolved fluorescence). An increased fluorescent signal over background indicates a phosphorylation by polypeptide of the present invention or a molecule induced by polypeptide of the present invention.

Example 42: Assay for the Stimulation of Bone Marrow CD34+ Cell Proliferation.

This assay is based on the ability of human CD34+ to proliferate in the presence of

hematopoietic growth factors and evaluates the ability of isolated polypeptides expressed in mammalian cells to stimulate proliferation of CD34+ cells.

It has been previously shown that most mature precursors will respond to only a single signal. More immature precursors require at least two signals to respond. Therefore, to test the effect of polypeptides on hematopoietic activity of a wide range of progenitor cells, the assay contains a given polypeptide in the presence or absence of other hematopoietic growth factors. Isolated cells are cultured for 5 days in the presence of Stem Cell Factor (SCF) in combination with tested sample. SCF alone has a very limited effect on the proliferation of bone marrow (BM) cells, acting in such conditions only as a "survival" factor. However, combined with any factor exhibiting stimulatory effect on these cells (e.g., IL-3), SCF will cause a synergistic effect. Therefore, if the tested polypeptide has a stimulatory effect on a hematopoietic progenitors, such activity can be easily detected. Since normal BM cells have a low level of cycling cells, it is likely that any inhibitory effect of a given polypeptide, or agonists or antagonists thereof, might not be detected. Accordingly, assays for an inhibitory effect on progenitors is preferably tested in cells that are first subjected to *in vitro* stimulation with SCF+IL+3, and then contacted with the compound that is being evaluated for inhibition of such induced proliferation.

Briefly, CD34+ cells are isolated using methods known in the art. The cells are thawed and resuspended in medium (QBSF 60 serum-free medium with 1% L-glutamine (500ml) Quality Biological, Inc., Gaithersburg, MD Cat# 160-204-101). After several gentle centrifugation steps at 200 x g, cells are allowed to rest for one hour. The cell count is adjusted to 2.5×10^5 cells/ml. During this time, 100 μ l of sterile water is added to the peripheral wells of a 96-well plate. The cytokines that can be tested with a given polypeptide in this assay is rhSCF (R&D Systems, Minneapolis, MN, Cat# 255-SC) at 50 ng/ml alone and in combination with rhSCF and rhIL-3 (R&D Systems, Minneapolis, MN, Cat# 203-ML) at 30 ng/ml. After one hour, 10 μ l of prepared cytokines, 50 μ l of the supernatants prepared in Example 31 (supernatants at 1:2 dilution = 50 μ l) and 20 μ l of diluted cells are added to the media which is already present in the wells to allow for a final total volume of 100 μ l. The plates are then placed in a 37°C/5% CO₂ incubator for five days.

Eighteen hours before the assay is harvested, 0.5 μ Ci/well of [3H] Thymidine is added in a 10 μ l volume to each well to determine the proliferation rate. The experiment is terminated by harvesting the cells from each 96-well plate to a filtermat using the Tomtec

Harvester 96. After harvesting, the filtermats are dried, trimmed and placed into OmniFilter assemblies consisting of one OmniFilter plate and one OmniFilter Tray. 60 µl Microscint is added to each well and the plate sealed with TopSeal-A press-on sealing film. A bar code 15 sticker is affixed to the first plate for counting. The sealed plates is then loaded and the level of radioactivity determined via the Packard Top Count and the printed data collected for analysis. The level of radioactivity reflects the amount of cell proliferation.

The studies described in this example test the activity of a given polypeptide to stimulate bone marrow CD34+ cell proliferation. One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof. As a nonlimiting example, potential antagonists tested in this assay would be expected to inhibit cell proliferation in the presence of cytokines and/or to increase the inhibition of cell proliferation in the presence of cytokines and a given polypeptide. In contrast, potential agonists tested in this assay would be expected to enhance cell proliferation and/or to decrease the inhibition of cell proliferation in the presence of cytokines and a given polypeptide.

The ability of a gene to stimulate the proliferation of bone marrow CD34+ cells indicates that polynucleotides and polypeptides corresponding to the gene are useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections above, and elsewhere herein.

Example 43: Assay for Extracellular Matrix Enhanced Cell Response (EMECR).

The objective of the Extracellular Matrix Enhanced Cell Response (EMECR) assay is to identify gene products (e.g., isolated polypeptides) that act on the hematopoietic stem cells in the context of the extracellular matrix (ECM) induced signal.

Cells respond to the regulatory factors in the context of signal(s) received from the surrounding microenvironment. For example, fibroblasts, and endothelial and epithelial stem cells fail to replicate in the absence of signals from the ECM. Hematopoietic stem cells can undergo self-renewal in the bone marrow, but not in *in vitro* suspension culture. The ability of stem cells to undergo self-renewal *in vitro* is dependent upon their interaction with the

stromal cells and the ECM protein fibronectin (fn). Adhesion of cells to fn is mediated by the $\alpha_5\beta_1$ and $\alpha_4\beta_1$ integrin receptors, which are expressed by human and mouse hematopoietic stem cells. The factor(s) which integrate with the ECM environment and responsible for stimulating stem cell self-renewal has not yet been identified. Discovery of such factors should be of great interest in gene therapy and bone marrow transplant applications

Briefly, polystyrene, non tissue culture treated, 96-well plates are coated with fn fragment at a coating concentration of $0.2 \mu\text{g}/\text{cm}^2$. Mouse bone marrow cells are plated (1,000 cells/well) in 0.2 ml of serum-free medium. Cells cultured in the presence of IL-3 (5 ng/ml) + SCF (50 ng/ml) would serve as the positive control, conditions under which little self-renewal but pronounced differentiation of the stem cells is to be expected. Gene products of the invention (e.g., including, but not limited to, polynucleotides and polypeptides of the present invention, and supernatants produced in Example 31), are tested with appropriate negative controls in the presence and absence of SCF(5.0 ng/ml), where test factor supernates represent 10% of the total assay volume. The plated cells are then allowed to grow by incubating in a low oxygen environment (5% CO_2 , 7% O_2 , and 88% N_2) tissue culture incubator for 7 days. The number of proliferating cells within the wells is then quantitated by measuring thymidine incorporation into cellular DNA. Verification of the positive hits in the assay will require phenotypic characterization of the cells, which can be accomplished by scaling up of the culture system and using appropriate antibody reagents against cell surface antigens and FACScan.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

If a particular polypeptide of the present invention is found to be a stimulator of hematopoietic progenitors, polynucleotides and polypeptides corresponding to the gene encoding said polypeptide may be useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections above, and elsewhere herein. The gene product may also be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Additionally, the polynucleotides and/or polypeptides of the gene of interest and/or

agonists and/or antagonists thereof, may also be employed to inhibit the proliferation and differentiation of hematopoietic cells and therefore may be employed to protect bone marrow stem cells from chemotherapeutic agents during chemotherapy. This antiproliferative effect may allow administration of higher doses of chemotherapeutic agents and, therefore, more effective chemotherapeutic treatment.

Moreover, polynucleotides and polypeptides corresponding to the gene of interest may also be useful for the treatment and diagnosis of hematopoietic related disorders such as, for example, anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex-vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia.

Example 44: Human Dermal Fibroblast and Aortic Smooth Muscle Cell Proliferation.

The polypeptide of interest is added to cultures of normal human dermal fibroblasts (NHDF) and human aortic smooth muscle cells (AoSMC) and two co-assays are performed with each sample. The first assay examines the effect of the polypeptide of interest on the proliferation of normal human dermal fibroblasts (NHDF) or aortic smooth muscle cells (AoSMC). Aberrant growth of fibroblasts or smooth muscle cells is a part of several pathological processes, including fibrosis, and restenosis. The second assay examines IL6 production by both NHDF and SMC. IL6 production is an indication of functional activation. Activated cells will have increased production of a number of cytokines and other factors, which can result in a proinflammatory or immunomodulatory outcome. Assays are run with and without co-TNF α stimulation, in order to check for costimulatory or inhibitory activity.

Briefly, on day 1, 96-well black plates are set up with 1000 cells/well (NHDF) or 2000 cells/well (AoSMC) in 100 μ l culture media. NHDF culture media contains: Clonetics FB basal media, 1mg/ml hFGF, 5mg/ml insulin, 50mg/ml gentamycin, 2%FBS, while AoSMC culture media contains Clonetics SM basal media, 0.5 μ g/ml hEGF, 5mg/ml insulin, 1 μ g/ml hFGF, 50mg/ml gentamycin, 50 μ g/ml Amphotericin B, 5%FBS. After incubation at 37°C for at least 4-5 hours, culture media is aspirated and replaced with growth arrest media. Growth arrest media for NHDF contains fibroblast basal media, 50mg/ml gentamycin, 2%

FBS, while growth arrest media for AoSMC contains SM basal media, 50mg/ml gentamycin, 50µg/ml Amphotericin B, 0.4% FBS. Incubate at 37°C until day 2.

On day 2, serial dilutions and templates of the polypeptide of interest are designed such that they always include media controls and known-protein controls. For both
5 stimulation and inhibition experiments, proteins are diluted in growth arrest media. For inhibition experiments, TNFa is added to a final concentration of 2ng/ml (NHDF) or 5ng/ml (AoSMC). Add 1/3 vol media containing controls or polypeptides of the present invention and incubate at 37°C/5% CO₂ until day 5.

Transfer 60µl from each well to another labeled 96-well plate, cover with a plate-sealer, and store at 4°C until Day 6 (for IL6 ELISA). To the remaining 100 µl in the cell
10 culture plate, aseptically add Alamar Blue in an amount equal to 10% of the culture volume (10µl). Return plates to incubator for 3 to 4 hours. Then measure fluorescence with excitation at 530nm and emission at 590nm using the CytoFluor. This yields the growth stimulation/inhibition data.

On day 5, the IL6 ELISA is performed by coating a 96 well plate with 50-100 ul/well
15 of Anti-Human IL6 Monoclonal antibody diluted in PBS, pH 7.4, incubate ON at room temperature.

On day 6, empty the plates into the sink and blot on paper towels. Prepare Assay Buffer containing PBS with 4% BSA. Block the plates with 200 µl/well of Pierce Super
20 Block blocking buffer in PBS for 1-2 hr and then wash plates with wash buffer (PBS, 0.05% Tween-20). Blot plates on paper towels. Then add 50 µl/well of diluted Anti-Human IL-6 Monoclonal, Biotin-labeled antibody at 0.50 mg/ml. Make dilutions of IL-6 stock in media (30, 10, 3, 1, 0.3, 0 ng/ml). Add duplicate samples to top row of plate. Cover the plates and incubate for 2 hours at RT on shaker. Plates are washed with wash buffer and blotted on
25 paper towels. Dilute EU-labeled Streptavidin 1:1000 in Assay buffer, and add 100 µl/well. Cover the plate and incubate 1 h at RT. Plates are again washed with wash buffer and blotted on paper towels. Add 100 µl/well of Enhancement Solution and shake for 5 minutes. Read the plate on the Wallac DELFIA Fluorometer. Readings from triplicate samples in each assay are tabulated and averaged.

30 A positive result in this assay suggests AoSMC cell proliferation and that the polypeptide of the present invention may be involved in dermal fibroblast proliferation and/or smooth muscle cell proliferation. A positive result also suggests many potential uses of

polypeptides, polynucleotides, agonists and/or antagonists of the polynucleotide/polypeptide of the present invention which gives a positive result. For example, inflammation and immune responses, wound healing, and angiogenesis, as detailed throughout this specification. Particularly, polypeptides of the present invention and polynucleotides of the present invention may be used in wound healing and dermal regeneration, as well as the promotion of vasculogenesis, both of the blood vessels and lymphatics. The growth of vessels can be used in the treatment of, for example, cardiovascular diseases. Additionally, antagonists of polypeptides and polynucleotides of the invention may be useful in treating diseases, disorders, and/or conditions which involve angiogenesis by acting as an anti-vascular (e.g., anti-angiogenesis). These diseases, disorders, and/or conditions are known in the art and/or are described herein, such as, for example, malignancies, solid tumors, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; arteriosclerotic plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uveitis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophiliac joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis. Moreover, antagonists of polypeptides and polynucleotides of the invention may be useful in treating anti-hyperproliferative diseases and/or anti-inflammatory known in the art and/or described herein.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

Example 45: Cellular Adhesion Molecule (CAM) Expression on Endothelial Cells.

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Briefly, endothelial cells (e.g., Human Umbilical Vein Endothelial cells (HUVECs)) are grown in a standard 96 well plate to confluence, growth medium is removed from the cells and replaced with 100 μ l of 199 Medium (10% fetal bovine serum (FBS)). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10 μ l volumes). Plates are then incubated at 37°C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100 μ l of 0.1% paraformaldehyde-PBS(with Ca⁺⁺ and Mg⁺⁺) is added to each well. Plates are held at 4°C for 30 min. Fixative is removed from the wells and wells are washed 1X with PBS(+Ca,Mg) + 0.5% BSA and drained. 10 μ l of diluted primary antibody is added to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10 μ g/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed three times with PBS(+Ca,Mg) + 0.5% BSA. 20 μ l of diluted ExtrAvidin-Alkaline Phosphatase (1:5,000 dilution, referred to herein as the working dilution) are added to each well and incubated at 37°C for 30 min. Wells are washed three times with PBS(+Ca,Mg)+0.5% BSA. Dissolve 1 tablet of p-Nitrophenol Phosphate pNPP per 5 ml of glycine buffer (pH 10.4). 100 μ l of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphatase in glycine buffer: 1:5,000 (10^0) > $10^{-0.5}$ > 10^{-1} > $10^{-1.5}$. 5 μ l of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100 μ l of pNPP reagent is then added to each of the standard wells. The plate is incubated at 37°C for 4h. A volume of 50 μ l of 3M NaOH is added to all wells. The plate is read on a plate reader

at 405 nm using the background subtraction option on blank wells filled with glycine buffer only. Additionally, the template is set up to indicate the concentration of AP-conjugate in each standard well [5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

5

Example 46: Alamar Blue Endothelial Cells Proliferation Assay.

This assay may be used to quantitatively determine protein mediated inhibition of bFGF-induced proliferation of Bovine Lymphatic Endothelial Cells (LECs), Bovine Aortic
10 Endothelial Cells (BAECs) or Human Microvascular Uterine Myometrial Cells (UTMECs). This assay incorporates a fluorometric growth indicator based on detection of metabolic activity. A standard Alamar Blue Proliferation Assay is prepared in EGM-2MV with 10 ng /ml of bFGF added as a source of endothelial cell stimulation. This assay may be used with a variety of endothelial cells with slight changes in growth medium and cell concentration.
15 Dilutions of the protein batches to be tested are diluted as appropriate. Serum-free medium (GIBCO SFM) without bFGF is used as a non-stimulated control and Angiostatin or TSP-1 are included as a known inhibitory controls.

Briefly, LEC, BAECs or UTMECs are seeded in growth media at a density of 5000 to 2000 cells/well in a 96 well plate and placed at 37-C overnight. After the overnight
20 incubation of the cells, the growth media is removed and replaced with GIBCO EC-SFM. The cells are treated with the appropriate dilutions of the protein of interest or control protein sample(s) (prepared in SFM) in triplicate wells with additional bFGF to a concentration of 10 ng/ ml. Once the cells have been treated with the samples, the plate(s) is/are placed back in the 37° C incubator for three days. After three days 10 ml of stock alamar blue (Biosource
25 Cat# DAL1100) is added to each well and the plate(s) is/are placed back in the 37°C incubator for four hours. The plate(s) are then read at 530nm excitation and 590nm emission using the CytoFluor fluorescence reader. Direct output is recorded in relative fluorescence units.

Alamar blue is an oxidation-reduction indicator that both fluoresces and changes color
30 in response to chemical reduction of growth medium resulting from cell growth. As cells grow in culture, innate metabolic activity results in a chemical reduction of the immediate surrounding environment. Reduction related to growth causes the indicator to change from

oxidized (non-fluorescent blue) form to reduced (fluorescent red) form. i.e. stimulated proliferation will produce a stronger signal and inhibited proliferation will produce a weaker signal and the total signal is proportional to the total number of cells as well as their metabolic activity. The background level of activity is observed with the starvation medium alone. This is compared to the output observed from the positive control samples (bFGF in growth medium) and protein dilutions.

Example 47: Detection of Inhibition of a Mixed Lymphocyte Reaction.

This assay can be used to detect and evaluate inhibition of a Mixed Lymphocyte Reaction (MLR) by gene products (e.g., isolated polypeptides). Inhibition of a MLR may be due to a direct effect on cell proliferation and viability, modulation of costimulatory molecules on interacting cells, modulation of adhesiveness between lymphocytes and accessory cells, or modulation of cytokine production by accessory cells. Multiple cells may be targeted by these polypeptides since the peripheral blood mononuclear fraction used in this assay includes T, B and natural killer lymphocytes, as well as monocytes and dendritic cells.

Polypeptides of interest found to inhibit the MLR may find application in diseases associated with lymphocyte and monocyte activation or proliferation. These include, but are not limited to, diseases such as asthma, arthritis, diabetes, inflammatory skin conditions, psoriasis, eczema, systemic lupus erythematosus, multiple sclerosis, glomerulonephritis, inflammatory bowel disease, crohn's disease, ulcerative colitis, arteriosclerosis, cirrhosis, graft vs. host disease, host vs. graft disease, hepatitis, leukemia and lymphoma.

Briefly, PBMCs from human donors are purified by density gradient centrifugation using Lymphocyte Separation Medium (LSM[®], density 1.0770 g/ml, Organon Teknika Corporation, West Chester, PA). PBMCs from two donors are adjusted to 2×10^6 cells/ml in RPMI-1640 (Life Technologies, Grand Island, NY) supplemented with 10% FCS and 2 mM glutamine. PBMCs from a third donor is adjusted to 2×10^5 cells/ml. Fifty microliters of PBMCs from each donor is added to wells of a 96-well round bottom microtiter plate. Dilutions of test materials (50 μ l) is added in triplicate to microtiter wells. Test samples (of the protein of interest) are added for final dilution of 1:4; rhuIL-2 (R&D Systems, Minneapolis, MN, catalog number 202-IL) is added to a final concentration of 1 μ g/ml; anti-CD4 mAb (R&D Systems, clone 34930.11, catalog number MAB379) is added to a final

concentration of 10 µg/ml. Cells are cultured for 7-8 days at 37°C in 5% CO₂, and 1 µC of [³H] thymidine is added to wells for the last 16 hrs of culture. Cells are harvested and thymidine incorporation determined using a Packard TopCount. Data is expressed as the mean and standard deviation of triplicate determinations.

5 Samples of the protein of interest are screened in separate experiments and compared to the negative control treatment, anti-CD4 mAb, which inhibits proliferation of lymphocytes and the positive control treatment, IL-2 (either as recombinant material or supernatant), which enhances proliferation of lymphocytes.

One skilled in the art could easily modify the exemplified studies to test the activity of
10 polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

Example 48: Assays for Protease Activity.

15 The following assay may be used to assess protease activity of the colon or colon cancer related polypeptides of the invention.

Gelatin and casein zymography are performed essentially as described (Heusen et al., *Anal. Biochem.*, 102:196-202 (1980); Wilson et al., *Journal of Urology*, 149:653-658 (1993)). Samples are run on 10% polyacrylamide/0.1% SDS gels containing 1% gelatin or casein,
20 soaked in 2.5% triton at room temperature for 1 hour, and in 0.1M glycine, pH 8.3 at 37°C 5 to 16 hours. After staining in amido black areas of proteolysis appear as clear areas against the blue-black background. Trypsin (Sigma T8642) is used as a positive control.

Protease activity is also determined by monitoring the cleavage of n-a-benzoyl-L-arginine ethyl ester (BAEE) (Sigma B-4500. Reactions are set up in (25mM NaPO₄, 1mM
25 EDTA, and 1mM BAEE), pH 7.5. Samples are added and the change in adsorbance at 260nm is monitored on the Beckman DU-6 spectrophotometer in the time-drive mode. Trypsin is used as a positive control

Additional assays based upon the release of acid-soluble peptides from casein or hemoglobin measured as adsorbance at 280 nm or colorimetrically using the Folin method
30 are performed as described in Bergmeyer, et al., *Methods of Enzymatic Analysis*, 5 (1984). Other assays involve the solubilization of chromogenic substrates (Ward, *Applied Science*, 251-317 (1983).

Example 49: Identifying Serine Protease Substrate Specificity.

Methods known in the art or described herein may be used to determine the substrate
5 specificity of the polypeptides of the present invention having serine protease activity. A
preferred method of determining substrate specificity is by the use of positional scanning
synthetic combinatorial libraries as described in GB 2 324 529 (incorporated herein in its
entirety).

Example 50: Ligand Binding Assays.

The following assay may be used to assess ligand binding activity of the colon or
colon cancer related polypeptides of the invention.

Ligand binding assays provide a direct method for ascertaining receptor
15 pharmacology and are adaptable to a high throughput format. The purified ligand for a colon
or colon cancer related polypeptide is radiolabeled to high specific activity (50-2000
Ci/mmol) for binding studies. A determination is then made that the process of radiolabeling
does not diminish the activity of the ligand towards its colon or colon cancer related
polypeptide. Assay conditions for buffers, ions, pH and other modulators such as nucleotides
20 are optimized to establish a workable signal to noise ratio for both membrane and whole cell
colon or colon cancer related polypeptide sources. For these assays, specific colon or colon
cancer related polypeptide binding is defined as total associated radioactivity minus the
radioactivity measured in the presence of an excess of unlabeled competing ligand. Where
possible, more than one competing ligand is used to define residual nonspecific binding.

Example 51: Functional Assay in *Xenopus* Oocytes.

Capped RNA transcripts from linearized plasmid templates encoding the colon or
colon cancer related antigen cDNAs of the invention are synthesized in vitro with RNA
30 polymerases in accordance with standard procedures. In vitro transcripts are suspended in
water at a final concentration of 0.2 mg/ml. Ovarian lobes are removed from adult female
toads, Stage V defolliculated oocytes are obtained, and RNA transcripts (10 ng/oocyte) are

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injected in a 50 nl bolus using a microinjection apparatus. Two electrode voltage clamps are used to measure the currents from individual *Xenopus oocytes* in response to colon cancer antigen or colon cancer antigen agonist exposure. Recordings are made in Ca²⁺ free Barth's medium at room temperature. The *Xenopus* system can be used to screen
5 known ligands and tissue/cell extracts for activating ligands.

Example 52: Microphysiometric Assays.

Activation of a wide variety of secondary messenger systems results in extrusion of
10 small amounts of acid from a cell. The acid formed is largely as a result of the increased metabolic activity required to fuel the intracellular signaling process. The pH changes in the media surrounding the cell are very small but are detectable by the CYTOSENSOR microphysiometer (Molecular Devices Ltd., Menlo Park, Calif.). The CYTOSENSOR is thus capable of detecting the activation of a colon cancer antigen which is coupled to an energy
15 utilizing intracellular signaling pathway.

Example 53: Extract/Cell Supernatant Screening.

A large number of mammalian receptors exist for which there remains, as yet, no
20 cognate activating ligand (agonist). Thus, active ligands for these receptors may not be included within the ligands banks as identified to date. Accordingly, the colon cancer antigen of the invention can also be functionally screened (using calcium, cAMP, microphysiometer, oocyte electrophysiology, etc., functional screens) against tissue extracts to identify its natural ligands. Extracts that produce positive functional responses can be sequentially
25 subfractionated until an activating ligand is isolated identified.

Example 54: Calcium and cAMP Functional Assays.

Seven transmembrane receptors which are expressed in HEK 293 cells have been
30 shown to be coupled functionally to activation of PLC and calcium mobilization and/or cAMP stimulation or inhibition. Basal calcium levels in the HEK 293 cells in receptor-transfected or vector control cells were observed to be in the normal, 100 nM to 200 nM,

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range. HEK 293 cells expressing recombinant receptors are loaded with fura 2 and in a single day >150 selected ligands or tissue/cell extracts are evaluated for agonist induced calcium mobilization. Similarly, HEK 293 cells expressing recombinant receptors are evaluated for the stimulation or inhibition of cAMP production using standard cAMP
5 quantitation assays. Agonists presenting a calcium transient or cAMP fluctuation are tested in vector control cells to determine if the response is unique to the transfected cells expressing receptor.

Example 55: ATP-binding assay.

10

The following assay may be used to assess ATP-binding activity of the colon or colon cancer related polypeptides of the invention.

ATP-binding activity of the colon or colon cancer related polypeptides of the invention may be detected using the ATP-binding assay described in U.S. Patent 5, 858, 719,
15 which is herein incorporated by reference in its entirety. Briefly, ATP-binding to colon or colon cancer related polypeptides of the invention is measured via photoaffinity labeling with 8-azido-ATP in a competition assay. Reaction mixtures containing 1 mg/ml of the ABC transport protein of the present invention are incubated with varying concentrations of ATP, or the non-hydrolyzable ATP analog adenylyl-5'-imidodiphosphate for 10 minutes at 4°C. A
20 mixture of 8-azido-ATP (Sigma Chem. Corp., St. Louis, MO.) plus 8-azido-ATP (^{-32}P -ATP) (5 mCi/ μmol , ICN, Irvine CA.) is added to a final concentration of 100 μM and 0.5 ml aliquots are placed in the wells of a porcelain spot plate on ice. The plate is irradiated using a short wave 254 nm UV lamp at a distance of 2.5 cm from the plate for two one-minute intervals with a one-minute cooling interval in between. The reaction is stopped by addition
25 of dithiothreitol to a final concentration of 2mM. The incubations are subjected to SDS-PAGE electrophoresis, dried, and autoradiographed. Protein bands corresponding to the particular colon or colon cancer related polypeptides of the invention are excised, and the radioactivity quantified. A decrease in radioactivity with increasing ATP or adenylyl-5'-imidodiphosphate provides a measure of ATP affinity to the colon or colon cancer related
30 polypeptides.

Example 56: Small Molecule***Screening.***

This invention is particularly useful for screening therapeutic compounds by using the colon or colon cancer related polypeptides of the invention, or binding fragments thereof, in any of a variety of drug screening techniques. The polypeptide or fragment employed in such a test may be affixed to a solid support, expressed on a cell surface, free in solution, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide or fragment. Drugs are screened against such transformed cells in competitive binding assays. One may measure, for example, the formulation of complexes between the agent being tested and a colon or colon cancer related polypeptide of the invention.

Thus, the present invention provides methods of screening for drugs or any other agents which affect activities mediated by the colon or colon cancer related polypeptides of the invention. These methods comprise contacting such an agent with a colon or colon cancer related polypeptide of the invention or a fragment thereof and assaying for the presence of a complex between the agent and the colon or colon cancer related polypeptides or a fragment thereof, by methods well known in the art. In such a competitive binding assay, the agents to screen are typically labeled. Following incubation, free agent is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of a particular agent to bind to the colon or colon cancer related polypeptides of the invention.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the colon or colon cancer related polypeptides of the invention, and is described in great detail in European Patent Application 84/03564, published on September 13, 1984, which is herein incorporated by reference in its entirety. Briefly stated, large numbers of different small molecule test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The test compounds are reacted with colon or colon cancer related polypeptides of the invention and washed. Bound colon or colon cancer related polypeptides are then detected by methods well known in the art. Purified colon or colon cancer related polypeptides are coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies may be used to capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding colon or colon cancer related polypeptides of the invention specifically compete with a test compound for binding to the colon or colon cancer related polypeptides or fragments thereof. In this manner, the antibodies are used to
5 detect the presence of any peptide which shares one or more antigenic epitopes with a colon or colon cancer related polypeptides.

Example 57: Phosphorylation Assay.

10 In order to assay for phosphorylation activity of the colon or colon cancer related polypeptides of the invention, a phosphorylation assay as described in U.S. Patent 5,958,405 (which is herein incorporated by reference) is utilized. Briefly, phosphorylation activity may be measured by phosphorylation of a protein substrate using gamma-labeled ^{32}P -ATP and quantitation of the incorporated radioactivity using a gamma radioisotope counter. The colon
15 or colon cancer related polypeptides of the invention are incubated with the protein substrate, ^{32}P -ATP, and a kinase buffer. The ^{32}P incorporated into the substrate is then separated from free ^{32}P -ATP by electrophoresis, and the incorporated ^{32}P is counted and compared to a negative control. Radioactivity counts above the negative control are indicative of phosphorylation activity of the colon or colon cancer related polypeptides of the invention.

20

Example 58: Detection of Phosphorylation Activity (Activation) of Colon or Colon Cancer Related Polypeptides of the Invention in the Presence of Colon or Colon Cancer Related Polypeptides Ligands.

25 Methods known in the art or described herein may be used to determine the phosphorylation activity of the colon or colon cancer related polypeptides of the invention. A preferred method of determining phosphorylation activity is by the use of the tyrosine phosphorylation assay as described in US 5,817,471 (incorporated herein by reference).

30 ***Example 59: Identification Of Signal Transduction Proteins That Interact With Colon or Colon Cancer Related Polypeptides Of The Present Invention.***

The inventive purified colon or colon cancer related polypeptides of the invention are research tools for the identification, characterization and purification of additional signal transduction pathway proteins or receptor proteins. Briefly, labeled receptor PTK polypeptide is useful as a reagent for the purification of molecules with which it interacts. In one embodiment of affinity purification, receptor PTK polypeptide is covalently coupled to a chromatography column. Cell-free extract derived from putative target cells, such as carcinoma tissues, is passed over the column, and molecules with appropriate affinity bind to the receptor PTK polypeptides, or specific phosphotyrosine-recognition domains thereof. The receptor PTK polypeptide interacting protein-complex is recovered from the column, dissociated, and the recovered molecule subjected to N-terminal protein sequencing. This amino acid sequence is then used to identify the captured molecule or to design degenerate oligonucleotide probes for cloning the relevant gene from an appropriate cDNA library.

Example 60: IL-6 Bioassay.

To test the proliferative effects of the colon or colon cancer related polypeptides of the invention, the IL-6 Bioassay as described by Marz *et al.* is utilized (*Proc. Natl. Acad. Sci., U.S.A.*, 95:3251-56 (1998), which is herein incorporated by reference). Briefly, IL-6 dependent B9 murine cells are washed three times in IL-6 free medium and plated at a concentration of 5,000 cells per well in 50 μ l, and 50 μ l of the IL-6-like polypeptide is added. After 68 hrs. at 37°C, the number of viable cells is measured by adding the tetrazolium salt thiazolyl blue (MTT) and incubating for a further 4 hrs. at 37°C. B9 cells are lysed by SDS and optical density is measured at 570 nm. Controls containing IL-6 (positive) and no cytokine (negative) are utilized. Enhanced proliferation in the test sample(s) relative to the negative control is indicative of proliferative effects mediated by colon or colon cancer related polypeptides of the invention.

Example 61: Support of Chicken Embryo Neuron Survival.

To test whether sympathetic neuronal cell viability is supported by the colon or colon cancer related polypeptides of the invention, the chicken embryo neuronal survival assay of Senaldi *et al* is utilized (*Proc. Natl. Acad. Sci., U.S.A.*, 96:11458-63 (1998), which is herein

incorporated by reference). Briefly, motor and sympathetic neurons are isolated from chicken embryos, resuspended in L15 medium (with 10% FCS, glucose, sodium selenite, progesterone, conalbumin, putrescine, and insulin; Life Technologies, Rockville, MD.) and Dulbecco's modified Eagles medium [with 10% FCS, glutamine, penicillin, and 25 mM
5 Hepes buffer (pH 7.2); Life Technologies, Rockville, MD.], respectively, and incubated at 37°C in 5% CO₂ in the presence of different concentrations of the inventive purified IL-6-like polypeptide, as well as a negative control lacking any cytokine. After 3 days, neuron survival is determined by evaluation of cellular morphology, and through the use of the colorimetric assay of Mosmann (Mossmann, T., *J. Immunol. Methods*, 65:55-63 (1983)). Enhanced
10 neuronal cell viability as compared to the controls lacking cytokine is indicative of the ability of the inventive purified IL-6-like polypeptide(s) to enhance the survival of neuronal cells.

Example 62: Assay for Phosphatase Activity.

15 The following assay may be used to assess serine/threonine phosphatase (PTPase) activity of the colon or colon cancer related polypeptides of the invention.

In order to assay for serine/threonine phosphatase (PTPase) activity, assays can be utilized which are widely known to those skilled in the art. For example, the serine/threonine phosphatase (PSPase) activity is measured using a PSPase assay kit from New England
20 Biolabs, Inc. Myelin basic protein (MyBP), a substrate for PSPase, is phosphorylated on serine and threonine residues with cAMP-dependent Protein Kinase in the presence of [γ -³²P]ATP. Protein serine/threonine phosphatase activity is then determined by measuring the release of inorganic phosphate from ³²P-labeled MyBP.

Example 63: Interaction of Serine/Threonine Phosphatases with other Proteins.

The colon or colon cancer related polypeptides of the invention with serine/threonine phosphatase activity as determined in Example 62 are research tools for the identification,
30 characterization and purification of additional interacting proteins or receptor proteins, or other signal transduction pathway proteins. Briefly, a labeled colon or colon cancer related polypeptides of the invention is useful as a reagent for the purification of molecules with

which it interacts. In one embodiment of affinity purification, colon or colon cancer related polypeptides of the invention is covalently coupled to a chromatography column. Cell-free extract derived from putative target cells, such as neural or liver cells, is passed over the column, and molecules with appropriate affinity bind to the colon or colon cancer related polypeptides of the invention. The colon or colon cancer related polypeptides of the invention-complex is recovered from the column, dissociated, and the recovered molecule subjected to N-terminal protein sequencing. This amino acid sequence is then used to identify the captured molecule or to design degenerate oligonucleotide probes for cloning the relevant gene from an appropriate cDNA library.

Example 64: Assaying for Heparanase Activity.

In order to assay for heparanase activity of the colon or colon cancer related polypeptides of the invention, the heparanase assay described by Vlodavsky et al is utilized (Vlodavsky, I., et al., Nat. Med., 5:793-802 (1999)). Briefly, cell lysates, conditioned media or intact cells (1×10^6 cells per 35-mm dish) are incubated for 18 hrs at 37°C, pH 6.2-6.6, with ^{35}S -labeled ECM or soluble ECM derived peak I proteoglycans. The incubation medium is centrifuged and the supernatant is analyzed by gel filtration on a Sepharose CL-6B column (0.9 x 30 cm). Fractions are eluted with PBS and their radioactivity is measured. Degradation fragments of heparan sulfate side chains are eluted from Sepharose 6B at $0.5 < K_{av} < 0.8$ (peak II). Each experiment is done at least three times. Degradation fragments corresponding to "peak II," as described by Vlodavsky et al., is indicative of the activity of the colon or colon cancer related polypeptides of the invention in cleaving heparan sulfate.

Example 65: Immobilization of biomolecules.

This method provides a method for the stabilization of colon or colon cancer related polypeptides of the invention in non-host cell lipid bilayer constructs (see, e.g., Bieri et al., Nature Biotech 17:1105-1108 (1999), hereby incorporated by reference in its entirety herein) which can be adapted for the study of colon or colon cancer related polypeptides of the invention in the various functional assays described above. Briefly, carbohydrate-specific chemistry for biotinylation is used to confine a biotin tag to the extracellular domain of the

colon or colon cancer related polypeptides of the invention, thus allowing uniform orientation upon immobilization. A 50uM solution of colon or colon cancer related polypeptides of the invention in washed membranes is incubated with 20 mM NaIO₄ and 1.5 mg/ml (4mM) BACH or 2 mg/ml (7.5mM) biotin-hydrazide for 1 hr at room temperature
5 (reaction volume, 150ul). Then the sample is dialyzed (Pierce Slidealizer Cassett, 10 kDa cutoff; Pierce Chemical Co., Rockford IL) at 4C first for 5 h, exchanging the buffer after each hour, and finally for 12 h against 500 ml buffer R (0.15 M NaCl, 1 mM MgCl₂, 10 mM sodium phosphate, pH7). Just before addition into a cuvette, the sample is diluted 1:5 in buffer ROG50 (Buffer R supplemented with 50 mM octylglucoside).

10

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

15

The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other disclosures) in the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference. Further, the hard copy of the sequence listing submitted herewith and the corresponding computer readable form are both incorporated herein by reference in their
20 entireties. Moreover, the hard copy of and the corresponding computer readable form of the Sequence Listing of U.S. Patent Application Serial No. 60/157,137 and 60/163,280 are also incorporated herein by reference in its entirety.

Applicant's or agent's file reference number	PA005PCT	International application No.	UNASSIGNED
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

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A. The indications made below relate to the microorganism referred to in the description on page <u>311</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 20 May 1997	Accession Number 209059
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC). Continued on the Attached Pages 2 & 3	
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ATCC Deposit No. 209059**Page 2****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

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FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No. 209059

Page 3

DENMARK

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SWEDEN

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NETHERLANDS

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Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 20 May 1997	Accession Number 209060
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC). Continued on the Attached Pages 2 & 3	
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ATCC Deposit No. 209060**Page 2****CANADA**

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NORWAY

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AUSTRALIA

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FINLAND

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UNITED KINGDOM

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ATCC Deposit No. 209060**Page 3****DENMARK**

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SWEDEN

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NETHERLANDS

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B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 20 May 1997	Accession Number 209061
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC). Continued on the Attached Pages 2 & 3	
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ATCC Deposit No. 209061**Page 2****CANADA**

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FINLAND

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DENMARK

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B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 20 May 1997	Accession Number 209062
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States) Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC). Continued on the Attached Pages 2 & 3	
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ATCC Deposit No. 209062**Page 2****CANADA**

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AUSTRALIA

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FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No. 209062**Page 3****DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	PA005PCT	International application No.	UNASSIGNED
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13 bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>311</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 20 May 1997	Accession Number 209063
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC). Continued on the Attached Pages 2 & 3	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

For receiving Office use only	For International Bureau use only
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

ATCC Deposit No. 209063**Page 2****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

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FINLAND

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UNITED KINGDOM

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ATCC Deposit No. 209063

Page 3

DENMARK

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SWEDEN

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NETHERLANDS

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Applicant's or agent's file reference number	PA005PCT	International application No.	UNASSIGNED
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13 bis)

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B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 20 May 1997	Accession Number 209064
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC). Continued on the Attached Pages 2 & 3	
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Authorized officer	Authorized officer

ATCC Deposit No. 209064**Page 2****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

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AUSTRALIA

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FINLAND

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UNITED KINGDOM

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ATCC Deposit No. 209064

Page 3

DENMARK

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SWEDEN

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NETHERLANDS

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Applicant's or agent's file reference number	PA005PCT	International application No.	UNASSIGNED
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13 bis)

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B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 20 May 1997	Accession Number 209065
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC). Continued on the Attached Pages 2 & 3	
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Authorized officer	Authorized officer

ATCC Deposit No. 209065**Page 2****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

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AUSTRALIA

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FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

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ATCC Deposit No. 209065

Page 3

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

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NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	PA005PCT	International application No.	UNASSIGNED
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13 bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>311</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution <u>American Type Culture Collection</u>	
Address of depositary institution (including postal code and country) <u>10801 University Boulevard</u> <u>Manassas, Virginia 20110-2209</u> <u>United States of America</u>	
Date of deposit <u>20 May 1997</u>	Accession Number <u>209066</u>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC). Continued on the Attached Pages 2 & 3	
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For receiving Office use only <input type="checkbox"/> This sheet was received with the international application Authorized officer	For International Bureau use only <input type="checkbox"/> This sheet was received by the International Bureau on: Authorized officer
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ATCC Deposit No. 209066**Page 2****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

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FINLAND

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UNITED KINGDOM

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ATCC Deposit No. 209066**Page 3****DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

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NETHERLANDS

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Applicant's or agent's file reference number	PA005PCT	International application No.	UNASSIGNED
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13 bis)

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B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 20 May 1997	Accession Number 209067
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC). Continued on the Attached Pages 2 & 3	
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For receiving Office use only <input type="checkbox"/> This sheet was received with the international application Authorized officer	For International Bureau use only <input type="checkbox"/> This sheet was received by the International Bureau on: Authorized officer
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ATCC Deposit No. 209067**Page 2****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

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AUSTRALIA

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FINLAND

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UNITED KINGDOM

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ATCC Deposit No. 209067

Page 3

DENMARK

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Applicant's or agent's file reference number	PA005PCT	International application No.	UNASSIGNED
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13 bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>311</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 20 May 1997	Accession Number 209068
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC). Continued on the Attached Pages 2 & 3	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

For receiving Office use only <input type="checkbox"/> This sheet was received with the international application Authorized officer	For International Bureau use only <input type="checkbox"/> This sheet was received by the International Bureau on: Authorized officer
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ATCC Deposit No. 209068**Page 2****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No. 209068**Page 3****DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	PA005PCT	International application No.	UNASSIGNED
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13 bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>311</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 20 May 1997	Accession Number 209069
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC). Continued on the Attached Pages 2 & 3	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

For receiving Office use only	For International Bureau use only
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

ATCC Deposit No. 209069**Page 2****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

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FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No. 209069

Page 3

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

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NETHERLANDS

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Applicant's or agent's file reference number	PA005PCT	International application No.	UNASSIGNED
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13 bis)

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B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 12 January 1998	Accession Number 209579
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC). Continued on the Attached Pages 2 & 3	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

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Authorized officer	Authorized officer

ATCC Deposit No. 209579**Page 2****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

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FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No. 209579

Page 3

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	PA005PCT	International application No.	UNASSIGNED
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13 bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>311</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 12 January 1998	Accession Number 209578
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States) Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC). Continued on the Attached Pages 2 & 3	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

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ATCC Deposit No. 209578**Page 2****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No. 209578**Page 3****DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	PA005PCT	International application No.	UNASSIGNED
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13 bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>311</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 16 July 1998	Accession Number 203067
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States) Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC). Continued on the Attached Pages 2 & 3	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g. "Accession Number of Deposit")	
For receiving Office use only <input type="checkbox"/> This sheet was received with the international application Authorized officer	For International Bureau use only <input type="checkbox"/> This sheet was received by the International Bureau on: Authorized officer

ATCC Deposit No. 203067**Page 2****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

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AUSTRALIA

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FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No. 203067

Page 3

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

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NETHERLANDS

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Applicant's or agent's file reference number	PA005PCT	International application No.	UNASSIGNED
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13 bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>311</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 16 July 1998	Accession Number 203068
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC). Continued on the Attached Pages 2 & 3	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

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ATCC Deposit No. 203068**Page 2****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

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AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No. 203068**Page 3****DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	PA005PCT	International application No.	UNASSIGNED
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13 bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>311</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 01 February 1999	Accession Number 203609
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC). Continued on the Attached Pages 2 & 3	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

For receiving Office use only	For International Bureau use only
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

ATCC Deposit No. 203609**Page 2****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

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FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No. 203609

Page 3

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

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NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	PA005PCT	International application No.	UNASSIGNED
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13 bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>311</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution <u>American Type Culture Collection</u>	
Address of depositary institution (including postal code and country) <u>10801 University Boulevard</u> <u>Manassas, Virginia 20110-2209</u> <u>United States of America</u>	
Date of deposit <u>01 February 1999</u>	Accession Number <u>203610</u>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC). Continued on the Attached Pages 2 & 3	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

For receiving Office use only <input type="checkbox"/> This sheet was received with the international application Authorized officer	For International Bureau use only <input type="checkbox"/> This sheet was received by the International Bureau on: Authorized officer
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ATCC Deposit No. 203610**Page 2****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

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FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No. 203610

Page 3

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	PA005PCT	International application No.	UNASSIGNED
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13 bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>311</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution <u>American Type Culture Collection</u>	
Address of depositary institution (including postal code and country) <u>10801 University Boulevard</u> <u>Manassas, Virginia 20110-2209</u> <u>United States of America</u>	
Date of deposit <u>17 November 1998</u>	Accession Number <u>203485</u>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC). Continued on the Attached Pages 2 & 3	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
For receiving Office use only <input type="checkbox"/> This sheet was received with the international application Authorized officer	For International Bureau use only <input type="checkbox"/> This sheet was received by the International Bureau on: Authorized officer

ATCC Deposit No. 203485**Page 2****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No. 203485**Page 3****DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

2212

Applicant's or agent's file reference number	PA005PCT	International application No.	UNASSIGNED
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13 bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>311</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 18 June 1999	Accession Number PTA-252
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC). Continued on the Attached Pages 2 & 3	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

For receiving Office use only	For International Bureau use only
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

ATCC Deposit No. PTA-252**Page 2****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

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FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No. PTA-252

Page 3

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	PA005PCT	International application No.	UNASSIGNED
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13 bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>311</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution <u>American Type Culture Collection</u>	
Address of depositary institution (including postal code and country) <u>10801 University Boulevard</u> <u>Manassas, Virginia 20110-2209</u> <u>United States of America</u>	
Date of deposit <u>18 June 1999</u>	Accession Number <u>PTA-253</u>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC). Continued on the Attached Pages 2 & 3	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

For receiving Office use only <input type="checkbox"/> This sheet was received with the international application Authorized officer	For International Bureau use only <input type="checkbox"/> This sheet was received by the International Bureau on: Authorized officer
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ATCC Deposit No. PTA-253**Page 2****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

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AUSTRALIA

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FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

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ATCC Deposit No. PTA-253
Page 3

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	PA005PCT	International application No.	UNASSIGNED
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13 bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>311</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 28 October 1999	Accession Number PTA-881
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC). Continued on the Attached Pages 2 & 3	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

For receiving Office use only	For International Bureau use only
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

ATCC Deposit No. PTA-881**Page 2****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No. PTA-881**Page 3****DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	PA005PCT	International application No.	UNASSIGNED
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13 bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>311</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 28 October 1999	Accession Number PTA-882
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC). Continued on the Attached Pages 2 & 3	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

For receiving Office use only <input type="checkbox"/> This sheet was received with the international application Authorized officer	For International Bureau use only <input type="checkbox"/> This sheet was received by the International Bureau on: Authorized officer
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ATCC Deposit No. PTA-882**Page 2****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

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AUSTRALIA

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FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

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ATCC Deposit No. PTA-882

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DENMARK

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SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

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What Is Claimed Is:

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a polynucleotide fragment of SEQ ID NO:X which is hybridizable to SEQ ID NO:X;

(b) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:Y which is hybridizable to SEQ ID NO:X;

(c) a polynucleotide encoding a polypeptide domain of SEQ ID NO:Y which is hybridizable to SEQ ID NO:X;

(d) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:Y which is hybridizable to SEQ ID NO:X;

(e) a polynucleotide encoding a polypeptide of SEQ ID NO:Y which is hybridizable to SEQ ID NO:X, having biological activity;

(f) a polynucleotide which is a variant of SEQ ID NO:X;

(g) a polynucleotide which is an allelic variant of SEQ ID NO:X;

(h) a polynucleotide which encodes a species homologue of the SEQ ID NO:Y;

(i) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(h), wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A residues or of only T residues.

2. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding a protein.

3. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:Y, which is hybridizable to SEQ ID NO:X.

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4. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:X, which is hybridizable to SEQ ID NO:X.

5. The isolated nucleic acid molecule of claim 2, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

6. The isolated nucleic acid molecule of claim 3, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

7. A recombinant vector comprising the isolated nucleic acid molecule of claim 1.

8. A method of making a recombinant host cell comprising the isolated nucleic acid molecule of claim 1.

9. A recombinant host cell produced by the method of claim 8.

10. The recombinant host cell of claim 9 comprising vector sequences.

11. An isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

- (a) a polypeptide fragment of SEQ ID NO:Y;
- (b) a polypeptide fragment of SEQ ID NO:Y, having biological activity;
- (c) a polypeptide domain of SEQ ID NO:Y;
- (d) a polypeptide epitope of SEQ ID NO:Y;
- (e) a full length protein of SEQ ID NO:Y;
- (f) a variant of SEQ ID NO:Y;
- (g) an allelic variant of SEQ ID NO:Y; or
- (h) a species homologue of the SEQ ID NO:Y.

12. The isolated polypeptide of claim 11, wherein the full length protein comprises sequential amino acid deletions from either the C-terminus or the N-terminus.

13. An isolated antibody that binds specifically to the isolated polypeptide of claim 11.

14. A recombinant host cell that expresses the isolated polypeptide of claim 11.

15. A method of making an isolated polypeptide comprising:
(a) culturing the recombinant host cell of claim 14 under conditions such that said polypeptide is expressed; and
(b) recovering said polypeptide.

16. The polypeptide produced by claim 15.

17. A method for preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of the polypeptide of claim 11 or the polynucleotide of claim 1.

18. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:
(a) determining the presence or absence of a mutation in the polynucleotide of claim 1; and
(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or absence of said mutation.

19. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

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(a) determining the presence or amount of expression of the polypeptide of claim 11 in a biological sample; and

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.

20. A method for identifying a binding partner to the polypeptide of claim 11 comprising:

(a) contacting the polypeptide of claim 11 with a binding partner; and

(b) determining whether the binding partner effects an activity of the polypeptide.

21. The gene corresponding to the cDNA sequence of SEQ ID NO:Y.

22. A method of identifying an activity in a biological assay, wherein the method comprises:

(a) expressing SEQ ID NO:X in a cell;

(b) isolating the supernatant;

(c) detecting an activity in a biological assay; and

(d) identifying the protein in the supernatant having the activity.

23. The product produced by the method of claim 20.